EFFECTS OF FEEDING MONENSIN ON LUTEINIZING HORMONE RESPONSE TO GONADOTROPIN RELEASING HORMONE OR ESTRADIOL-17β INJECTION, AND ON REPRODUCTIVE PERFORMANCE OF POSTPARTUM SUCKLED BEEF COWS

By

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The main objective of this experiment was to evaluate monensin effects on LH response of anestrous suckled beef cows. Hypothalamic and pituitary responsiveness was evaluated by monitoring plasma LH after injections of estradiol-17β (E_2, 10 mg, i.m.) and GnRH (100 ug, i.v.), respectively. A second objective was to evaluate monensin and hormonal effects on postpartum responses such as plasma concentrations of P_4 and glucose, VFA concentrations, ovarian structures, cervical and uterine dimensions, uterine location and tonus, and interval to first estrus. All cows were fed individually, based on metabolic weight according to NRC (1976), a supplement (16% CP, 85% TDN) from parturition to day 90 postpartum. One-half of the cows (14) received 200 mg of monensin with their daily supplement from days 10 to 90 postpartum. The cows were group fed daily an average of 8.6 kg of hay (4.5% CP) on pasture. Blood sampling and palpation of the reproductive tract were performed at 3-day intervals. Cows were challenged with E_2 or GnRH at 41 ± 1.5 days postpartum.

Monensin treated cows had a smaller and more gradual decrease in glucose concentrations than controls ($P < .05$). Monensin altered VFA concentrations by increasing propionate ($P < .01$) and by decreasing acetate ($P < .01$) and acetate:propionate ratio ($P < .01$). Gross uterine and cervical involution did not differ between treatments. Monensin treated cows had larger ovaries and more follicles than control cows. Mean concentrations of P_4 (< 1 ng/ml) and LH increased in all cows during the first 38 days postpartum. Plasma P_4 concentrations were not affected by monensin. Monensin treated cows injected with GnRH released less LH ($P < .01$) than controls. Luteinizing hormone response to E_2 could not be evaluated due to inadequate length of blood sampling (30 h). Fifty percent of the cows in each hormonal group had P_4 above 1 ng/ml for up to 6 days after hormone injection. Cows treated with GnRH secreted more P_4 than cows treated with E_2 ($P < .01$).

In conclusion, monensin induced changes in plasma glucose concentrations probably by altering ruminal VFA concentrations. Monensin may have a direct ovarian effect since ovarian size was altered within a few days after treatment started. Monensin had no effect on initiation of postpartum estrous cycles.

INTRODUCTION

Various factors influence the interval from parturition to first estrus in cattle (Casida et al., 1968; Wiltbank, 1970; Dunn and Kaltenbach, 1980; Wettemann, 1980). Probably the most important factor is the nutritional status of cows before and (or) after parturition (Wiltbank et al., 1962; Lamond, 1970a; Wiltbank, 1970; Dunn and Kaltenbach, 1980). An additional influence is suckling effect of the calf (Casida et al., 1968; Edgerton, 1980).

A drastic physiological adjustment occurs in the cow during the transition from pregnancy to lactation. Progestin concentrations decrease before parturition, whereas estrogens and corticoids increase until parturition and then undergo a rapid decrease (Smith et al., 1973). Edgerton and Hafs (1973) and Eley et al. (1981) observed these same patterns of change for progesterone (P_4) and estrogens and also reported that prolactin concentrations increased near parturition and luteinizing hormone (LH) concentrations were suppressed.

Regulation of nutrient partitioning during pregnancy involves homeorhetic controls arising from the conceptus which insures growth of the conceptus and gravid uterus as well as development of the mammary gland (Bauman and Currie, 1980). With the onset of lactation, many maternal tissues undergo further adaptations to support lactation. One of the major changes occurs in adipose tissue. Uptake of nutrients for synthesis of lipid pools is decreased and lipid reserves are mobilized via lipolysis. Another key nutrient is glucose. The maximally secreting mammary gland may require up to 80% of the total glucose turnover. This demand is met by increasing feed intake and rates of gluconeogenesis and glycogenolysis in the liver. Furthermore, nitrogen balance studies have demonstrated the importance of labile protein reserves in meeting amino acid needs for milk protein and glucose synthesis in early lactation (Bauman and Currie, 1980).

Restoration of normal ovarian cycles after parturition is a process that requires time for the uterus and endocrine system to restore their normal functions. This adjustment depends on the nutritional status of the animal and generally takes longer in suckled cows.

Responsiveness of the pituitary gland to GnRH is restored between days 7 and 14 in the postpartum period of milked cows (Britt et al., 1974; Fernandes et al., 1978a) and at 15-16 days postpartum in suckled beef cows (Irvin et al., 1981). In a study with suckled beef cows that were injected once with 500 ug of cloprostenol plus 500 ug of estradiol 28 h later, Nancarrow et al. (19/7) observed that most of the cows that were injected either between 21 and 30 days or between 31 and 73 days postpartum had shown estrus and a LH surge due to treatment. Very few cows had shown estrus and LH surge before 20 days postpartum due to injections of cloprostenol plus estradiol benzoate. In a similar experiment, Radford et al. (1978) used suckled and nonsuckled beef cows fed a mixture of 60% chopped lucerne hay and 40% rolled oats ad libitum. Postpartum ovarian activity was monitored by rectal palpation once a week and by blood P_4 concentrations of samples taken twice a week. After demonstrating that ovarian activity was reduced in suckled compared to nonsuckled cows, the cows were then injected about 40 days postpartum with cloprostenol and 27 h later with estradiol benzoate (500 ug; i.m.). Blood samples for determination of LH concentrations were collected at -27, -20, -3, 4, 18, 21, 23, 27, 30 and 45 h from the time of estradiol benzoate injection (0 h). Most of the suckled cows failed to release LH (6/7) and express estrus (7/7) in response to the treatment, whereas all seven of the nonsuckled cows were cycling by 40 days postpartum and only two cows did not respond to the cloprostenol and estradiol benzoate injections with luteal regression and estrous behavior. An LH surge occurred in four of the five nonsuckled cows that came into estrus. Suckled cows had lower LH concentrations than nonsuckled cows during the first 30 days postpartum. This was considered to be due to a reduced hypothalamic-pituitary function. Smith et al. (1981) worked with 15 pairs of identical twin dairy cows (one member of the twin pair was suckled by four calves and the other was dried-off just after parturition) to examine the response to 500 ug of estradiol-17β (E_2) injection i.m. 27.5 h after an injection of cloprostenol (500 ug i.m.). The presence of a LH response to this treatment was examined in five pairs of twins at weeks 1 and 7 after parturition, in another five pairs at weeks 3 and 9 and in a third group of five twin pairs at weeks 5 and 11. Suckled and nonlactating dairy cows were bled at 30 min intervals for 10 h starting 10 h after E_2 injection. Suckling was not allowed during the blood sampling period. A LH response to E_2 was not observed in suckled cows within 24 h until 7 weeks postpartum, but a response in some nonsuckled cows was recorded at weeks 1 and 5 after parturition. Smith et al. (1981) also reported that suckled cows took 2 weeks longer to restore LH response to luteinizing hormone release hormone (LHRH) than cows milked twice a day. Cows were injected with LHRH within 72 h after parturition and at intervals of 2 weeks for a period of 12 weeks. Calves were not allowed to suckle during the bleeding time for the 12 weeks of experimental period. There was a linear increase in the LH response to LHRH from parturition to 6 weeks postpartum in the milked cows. The development of the LH response was delayed in the suckled cows, and at 2 weeks postpartum the response was lower ($P < .01$) than that shown by the milked cows. Thus, the early restoration of the pituitary responsiveness to GnRH (LHRH), during the postpartum period, indicates that the pituitary is not responsible for the delay in the LH response to estrogen observed in suckled cows as compared to milked or nonlactating cows. Smith et al. (1981) pointed out that in suckled cows it appears that there is a longer delay postpartum in the development of a positive LH feedback to estrogen than in the capability to release LH in response to LHRH. They concluded that this may well be the dominant factor affecting anestrus. Acosta et al. (1983), using ovariectomized cows, observed that normally suckled ovariectomized cows implanted with E_2 had a greater reduction in basal serum LH concentration than normally suckled ovariectomized cows, ovariectomized cows injected with E_2 and early weaned and ovariectomized cows early weaned. These results indicated that suckled cows had a greater sensitivity to the inhibitory effect of E_2 on serum LH concentration.

Kesner et al. (1981), using ovariectomized cows observed that injection of 1 mg of estradiol was able to induce initially a decrease in LH secretion followed by a LH surge between 12 to 24 h post injection. Injection of LHRH at 20 min intervals starting 2 or 8 h after estradiol or oil injection, induced an LH surge, but the amount of LH released due to LHRH starting 2 h post estradiol, was smaller than that due to LHRH injection, starting 8 h after estradiol. Ovariectomized cows injected with LHRH at 2 h after oil injection released less LH than cows injected with LHRH 2 or 8 h post estradiol. This indicates that estradiol initially decreases LH concentration probably by decreasing LHRH secretion below the threshold that will cause detectable LH release. This is supported by the observation that endogenous pulsatile release of LH is abolished for about 12 h following estradiol injection. This initial decrease in LH after estradiol also could partially be due to a decrease in sensitivity of the pituitary to LHRH, since LHRH given at 20 min intervals, starting 2 h post estradiol released less LH than starting 8 h after estradiol injection. However, Kesner et al. (1981) also reported that estradiol increased the capacity of the pituitary to release LH in response to LHRH, based on the observation that injections of LHRH 2 h after oil injection released less LH than 2 h after estradiol injection in ovariectomized cows. It was suggested also that estradiol induced an LH surge in cows by increasing LHRH secretion at the time of the LH surge. Radford and Wallace (1974) reported that ovariectomized ewes under anesthesia with sodium pentobarbitone did not have an LH surge after an injection of estradiol benzoate, as observed in nonanesthetized ewes (16 to 24 h after injection of estradiol benzoate). However, anesthetized ovariectomized ewes were able to release LH after GnRH administration. They concluded that the stimulatory effect of estradiol benzoate on LH release in the ewe was mediated by the central nervous system.

Kesner et al. (1982) were able to induce LH and FSH surges in heifers by simultaneous ovariectomy and replacement therapy with estradiol and P_4 in amounts designed to mimic the normal transitions that occur during the proestrus period. Starting on day 11 to 15 of the estrous cycle, immediately after ovariectomy, the heifers were implanted with estradiol and infused intravenously with P_4 (3 mg/h). Hormone replacement was modified 24 h later to the following: 1, P_4 infusion was stopped and two estradiol implants were given at 12 h intervals for 36 h; 2, P_4 infusion was maintained and two estradiol implants were given at 12 h intervals for 36 h; 3, P_4 infusion and estradiol implant replacement were terminated and empty implants were given as in treatments 1 and 2. LH and FSH surges were induced in all heifers in treatment 1. No gonadotropin surges occurred in heifers of treatment 2 and 3 indicating that estrogen is stimulatory and progesterone inhibitory in the induction of LH and FSH surges.

The observation that an LH surge can be induced by injection of estradiol which increases LHRH release and pituitary sensitivity to LHRH (Kesner et al., 1981; 1982), that under anesthesia estradiol benzoate can not induce an LH surge in ewes but GnRH can (Radford and Wallace, 1974), and that the pituitary of the postpartum cows is responsive to GnRH (Britt et al., 1974; Fernandes et al., 1978a; Foster et al., 1980; Irvin et al., 1981), suggested that estrogen and LHRH injections could be utilized to differentiate between hypothalamic and pituitary factors limiting LH secretion in postpartum suckled beef cows.

It is not known at what level nutrition can affect reproduction in suckled cows, or whether nutrition influences hypothalamic and (or) pituitary control of LH. It is known, however, that an increase in the level of nutrition above the marginal level is followed frequently by better reproductive performance (Dunn and Kaltenbach, 1980).

Feed additives are commonly used in the cattle industry today. One of the additives is monensin, an ionophore. It has been shown that feeding monensin to cattle causes a change in the concentrations of propionate (increased), acetate (decreased) and acetate:propionate ratio (decreased; Richardson et al., 1976; Moseley et al., 1977; Turner et al., 1977). An increase in propionate production in the ruminant reduces loss of energy as methane and improves fermentation efficiency (Owens, 1980) and may reduce the animal heat increment (Blaxter and Wainman, 1964). These changes can contribute to the overall performance of the animal.

The main objective of this experiment was to observe the effects of feeding monensin on the LH response of anestrous suckled beef cows. Responsiveness of the hypothalamus and pituitary was evaluated by injections of E_2 and GnRH, respectively. A second objective was to evaluate the main effects of monensin and hormonal treatment (E_2 vs GnRH) on overall postpartum responses of suckled beef cows. These responses included plasma concentrations of P_4 and glucose, concentrations of ruminal volatile fatty acids (VFA), presence of ovarian structures (follicle and CL), cervical and uterine dimensions, uterine location and tonus, and interval to first detected estrus.

LITERATURE REVIEW

Uterine Involution

Observations that the first ovulation postpartum occurred more frequently in the ovary contralateral to the previously gravid uterine horn (Saiduddin et al., 1967; Foote and Peterson, 1968; Castenson, 1974; Guibault, personal communication) indicated that the uterus may influence early postpartum ovarian activity. The CL of pregnancy regresses by 4 to 7 days postpartum (Oxenreider, 1968; Wagner and Hansel, 1969). Mather and Melancon (1981) pointed out that the uterine-ovarian relationship is interdependent, and the health and integrity of the uterus can influence greatly ovarian activity and uterine repair. Improvement of the uterine environment can be enhanced by earlier ovarian activity during the postpartum period.

Uterine involution involves a reorganization of tissues and is comprised of three overlapping processes: 1, reduction in uterine size; 2, loss of uterine tissue; and 3, repair of residual tissue. Changes in the cervix are due mainly to reduction in size, by elimination of tissue fluid and some reduction in muscle tissue (Gier and Marion, 1968). The rapid decrease in dimensions of the postgravid uterine horn is due to vasoconstriction and muscular contractions. Shrinkage of the vascular system and muscular contractions continue to reduce the size of the postgravid uterus until it reaches a pregravid size by days 40 to 50 postpartum and normal involution processes are completed by 50 days postpartum. Morrow et al. (1966) observed that in dairy cows there was a marked decrease in uterine size and an increase in uterine tone from day 10 to 14 postpartum. Moller (1970) reported that the uterus and cervix involute at different rates. The cervix regressed at an even rate, whereas the uterine horns showed a sharp decrease, followed by a gradual decline.

Length of the postgravid uterine horn was reduced by half by 15 days postpartum and to one third by 30 days postpartum (Gier and Marion, 1968). Tennant and Peddicord (1968) agreed that the major portion of the involutionary process is completed during the first three weeks postpartum, and that reduction in uterine size is minimal after the fourth week. Morrow et al. (1966) and Marion et al. (1968), respectively, pointed out that regression of the uterus, as detected by palpation, was completed by 30 and 40 days postpartum.

Little change in uterine size was detected by rectal examination from days 30 to 60 postpartum (Morrow et al., 1966; Gier and Marion, 1968). Riesen et al. (1968) working with primiparous, suckled and nonlactating Holstein cows, and Lauderdale et al. (1968) working with suckled and nonlactating Hereford cows, observed that suckling accelerated uterine involution. Assessment of uterine involution was done after slaughter by weighing and measuring the uterus as well as by microscopic study of the uterine wall. Wiltbank and Cook (1958) estimated uterine involution by rectal palpation and reported that suckled Shorthorn cows took longer to have their uteri involuted than cows that were hand-milked twice a day (56 vs 44 day, $P < .01$). Moller (1970), however, did not detect significant differences in the speed of uterine involution between dairy cows milked and those suckled by 3 or 4 calves (31 vs 31 days). Likewise, Oxenreider (1968) detected no effect of suckling on uterine involution of beef cows. He also observed that uterine involution was not affected by induction of a CL after parturition or by ovariectomy. Marion et al. (1968), however, pointed out that ovariectomy significantly shortened the period of uterine regression in pluriparous cows. Daily injection of E_2 from day 3 to time of completed uterine involution did not influence significantly the rate of uterine regression. However, average interval to involution in treated cows was longer than that of controls and probably was due to a greater thickness of the uterine wall from E_2 stimulation (Marion et al., 1968). The previous gravid uterine horn decreased in size sooner when dairy cows were implanted with 100 ug of GnRH (Britt et al., 1974). They suggested that this may be due to increased P_4 secretion after GnRH administration. Fernandes et al. (1978b) also observed that GnRH injection enhanced the rate of uterine involution.

Postpartum Hormone Concentrations

Progesterone

Since P_4 is the major steroid synthesized by the CL, blood concentrations of P_4 are good indicators of luteal function (Wettemann, 1980). Progesterone concentration in the plasma of postpartum cows before cycling begins is lower than 1 ng/ml (Webb et al., 1977). No significant increases in P_4 concentrations are observed during postpartum anestrous for up to 18 days in dairy cows (Goodale et al., 1978; Webb et al., 1980) and 60 days in beef cows (Entwistle and Oga, 1977). Edgerton and Hafs (1973) and Fernandes et al. (1978a) reported an increase in P_4 concentrations during the postpartum period of dairy cows which corresponded to the occurrence of ovulation and CL formation as the postpartum period progressed (Morrow et al., 1966; Marion and Gier, 1968).

The initiation of cyclic activity, either in heifers or in postpartum cows, generally is preceded by an increase in P_4 concentration for a few days prior to first ovulation.

In prepuberal beef heifers, Gonzales-Padilla et al. (1975) observed two increases in P_4 before the first observed estrus. The first increase in P_4 lasted for 2 to 5 days and was followed by an LH peak (priming LH peak) which was followed by a second increase in P_4 that preceded the prepuberal LH peak. The source of P_4 was suggested to be the adrenal for the first rise and luteinized follicles for the second rise of P_4. However, Berardinelli et al. (1979) reported that the two transient increases in P_4 observed before puberty in beef heifers were the result of luteal tissue embedded within the ovary but not palpable or grossly observed on the ovarian surface.

During the postpartum period of dairy cows sampled from parturition to 40-78 days at 6 hour intervals (Schams et al., 1978), the first increase in P_4 concentration above 1 ng/ml usually was not of normal duration, reflecting either shortened or prolonged cycles. In more than half of the 25 animals studied, the first cycle was shortened by 6 to 7 days, and P_4 concentrations remained lower in the short cycles (4.5 ng/ml) compared to cows with normal estrous cycle length (6.7 ng/ml). Webb et al. (1980) reported that in 13 dairy cows milked twice a day, ovarian cyclic activity restarted by 16 days postpartum based on P_4 concentrations in blood collected every other day. Rectal palpation of the ovaries was carried out

7 days. Four additional cows under the same management were bled every 6 h from days 2 to 32 postpartum. A short-term elevation in plasma P_4 (duration of 9.6 days) in the first postpartum estrous cycle (14.9 days in length) occurred in seven of the 12 cows that had a second estrus and in three of the four cows there were more intensively bled. Surge of LH before the first elevation in P_4 was observed also and the time relationship between the occurrence of an LH surge and the increase in P_4 (4-5 days) was similar to that of the normal cycle. No CL was palpated during the rise in P_4, and it was suggested that developing follicles were the most likely source of P_4. The first postpartum estrous cycle, characterized by an increase in P_4 and palpation of a CL, was shorter than the second estrous cycle observed in 28 dairy cows, 17.7 vs 20.1 days, respectively (Stevenson and Britt, 1979).

Castenson (1974) observed an increase in concentrations of P_4 in nonsuckled primiparous beef heifers before cyclicity was restored. This increase was associated with CL formation in the absence of observable estrus. Cows were monitored daily by a P_4 radioimmunoassay and an increase in P_4 for two consecutive days was followed by ovariectomy for histological study. Suckled 2-year-old beef cows fed either 17.6 or 11.4 Mcal DE/day did not differ in P_4 concentration but both groups had an increase in P_4 before the first estrus postpartum (Corah et al., 1974). It was suggested that either luteinized follicles or the adrenal glands may be have been responsible for the preestrual P_4 secretion. The endocrine and ovarian changes in early postpartum suckled beef cows were studied by Kesler et al. (1980). Eight cows were bled at 30-min intervals for 4 h on days 7, 14, 21 and 28 postpartum and biweekly thereafter until restoration of ovarian cyclicity. Ovaries were examined via rectal palpation before each bleeding. An elevation of plasma P_4 in the absence of a CL was detected about one week prior to first postpartum estrus in six of the eight cows. Elevated P_4 concentrations and CL were detected in all cows during the first postpartum estrous cycle.

Using milk to determine P_4, Lamming et al. (1981) observed that 95% of the dairy cows had restored ovarian cyclicity by 50 days postpartum and that about 50% of the cows had an increase in milk P_4 ($>$ 3 ng/ml) for less than 10 days and estrus was frequently not observed until the end of the first complete cycle. In beef cows, the short initial postpartum cycles (milk P_4, $<$ 3 ng/ml for $<$ 10 days) occurred in 53% of the cows. Elevation in P_4 concentration also was observed by Rawlings et al. (1980). In their study, seven suckled Hereford cows were bled daily from parturition to 63-79 days postpartum and extra bleeding was conducted in six of the seven cows every 15 min for 8 h every 10 days. In five cows, in which the time of first estrus was accurately determined, an elevation in plasma P_4 for 3 to 5 days preceding first estrus was observed in four cows and followed estrus in one cow. Palpation of the ovaries did not clearly indicate occurrence of ovulation and formation of a CL until plasma P_4 concentrations were similar to a normal luteal phase. They suggested that luteinized follicles could be the source of P_4, since there were many large follicles present at the time of the early elevation of P_4. It was suggested also that, following parturition and under the suppressor effect of suckling, gonadotropin secretion appears to recover gradually in suckled beef cows and culminates in the luteinization of large ovarian follicles, or possibly in ovulation and formation of subnormal, short-lived corpora lutea. Humphrey et al. (1983) studied hormone concentrations in six second-calf, crossbred, suckled beef cows. Blood was collected at 6-h intervals from 14 days before parturition to 10 days after first postpartum estrus and intensively once a week at 15 min intervals for 4 hours. It was observed that the first postpartum estrus was associated with an increase in LH concentrations in serum and a transient increase in P_4 between 4.6 and .6 days before the first postpartum estrus occurred. A series of three experiments using beef cows suckled by a single calf once a day was carried out by Manns et al. (1983). In the first experiment, 12 cows were bled at 6-h intervals from day 25 until ovariectomy was performed in two cows each at 25, 27, 29, 31, 33 or 35 days postpartum. In the second experiment, cows were bled for 18 days at 6 h intervals beginning 25 days postpartum, and laparotomy for ovarian observation was performed on day 36 and repeated on day 50 postpartum. In the third experiment, seven cows were bled daily from parturition to 25 days postpartum. Peaks of serum LH and FSH preceded the first rise in P_4 which, in eight of nine cases, was due to the presence of CL that were functional for periods of 3 to 12 days. The other animal had a luteinized follicle. This indicates that the first ovulation in beef cows suckled by one calf is preceded by normal serum LH and FSH peaks and agrees with the study of Webb et al. (1980) in dairy cows milked twice a day and with the study of Humphrey et al. (1983) in beef cows suckled by one calf.

It is evident from these reports that in dairy cows (Schams et al., 1978; Stevenson and Britt, 1979; Webb et al., 1980; Lamming et al., 1981) and in beef cows (nonsuckled, Castenson, 1974; suckled, Corah et al., 1974; Kesler et al., 1980; Rawlings et al., 1980; Lamming et al., 1981; Manns et al., 1983) P_4 increases before the first normal postpartum estrous cycle and that this increase (> 1 ng/ml) is generally of shorter duration than the normal 14 days of subsequent cycles (Schams et al., 1978). An interesting point is the observation obtained by more frequent sampling in suckled beef cows (Humphrey et al., 1983; Manns et al., 1983) and dairy cows (Webb et al., 1980) that LH and FSH surges preceded the P_4 increase and that histologic studies have demonstrated that this initial P_4 increase is due to formation of a CL in the majority of cases of primiparous, nonlactating beef cows (Castenson, 1974) and in suckled beef cows (Manns et al., 1983). It is also interesting that prepubertal heifers have two surges in P_4 concentrations separated by an LH surge before the first estrus (Gonzales-Padilla et al., 1975; Berardinelli et al., 1979) and that these two transient increases in P_4 are due to luteal tissue embedded within the ovary but not palpable or visualized on the surface of the ovary (Berardinelli et al., 1979). Luteinized follicles also have been suggested as the source of the P_4 in the postpartum cow (Corah et al., 1974; Webb et al., 1980). Schams et al. (1978) suggested that this short early cycle was caused by inadequate levels of LH or its receptor. However, Lamming et al. (1981) were not able to support this theory related to lack of a luteotrophic factor and theorized that defects of follicular development lead to a reduction in steroidogenic capability of the subsequent luteal structure. This deficiency may be related to a lack of LH receptor activity.

Luteinizing Hormone

An increase in LH concentration up to 6 weeks postpartum was reported by Edgerton and Hafs (1973) in Holstein cows, while Goodale et al. (1978) observed that mean plasma concentration of LH in dairy cows increased from 2 to 13 days postpartum as did the daily maximum change and activity index of LH. Cows were bled hourly for 10 h every bleeding day (2 or 3, 7 or 8 and 12 or 13 days postpartum). An increase in plasma LH from 1 to 18 days postpartum in dairy cows was reported also (Kesler et al., 1977; Fernandes et al., 1978a; Jordan and Swanson, 1979; Kesler et al., 1980). Echternkamp (1978) suggested that the postpartum increase in LH secretion reflected an increase in LH content of the bovine pituitary, since it has been demonstrated that pituitary content of LH increased during the postpartum period (Saiduddin et al., 1968; Wagner et al., 1969). Humphrey et al. (1976) observed that the return to cyclic ovarian activity in postpartum cows was preceded by a constant increase in pulsatile releases of LH. An increase in frequency and magnitude of LH peaks for 10 to 33 days before the first elevation in plasma P_4 was reported by Rawlings et al. (1980) in beef cows. Lamming et al. (1981) observed that an increase in the basal concentration of LH occurred days before the first preovulatory surge of LH which preceded the first P_4 rise. Similarly, Humphrey et al. (1983) reported that serum concentrations of LH during the 4 weeks before estrus were higher ($P < .05$) than those observed 8 to 5 weeks before estrus. They also suggested that the increase in mean LH concentration from parturition to first estrus appeared to be the result of an increased frequency and magnitude of LH peaks during episodes of LH secretion. The postpartum increase in LH concentration also may be due to restoration of a positive feedback of estradiol on neuroendocrine centers (Stevenson and Britt, 1979).

Suckling Effect on Reproductive Performance

Suckling delayed first estrus when compared to milked cows (Wiltbank and Cook, 1958; Graves et al., 1968; Moller, 1970; Oxenreider and Wagner, 1971), nonsuckled and mastectomized cows (Short et al., 1972), and early weaned cows (Smith and Vincent, 1972). England et al. (1973) also reported that suckled beef cows had longer intervals from parturition to first ovulation ($P < .05$), and to first postpartum estrus ($P < .01$) than nonsuckled cows. Suckled cows also had a higher number of ovulations without estrous behavior ($P < .05$) and higher fertility at first estrus ($P < .01$) than nonsuckled cows. Stuedemann et al. (1981) reported that cows suckled once daily had a higher ($P < .05$) conception rate (90 vs 60%) and a shorter ($P < .05$) postpartum interval (83 vs 101 days) than cows suckled ad libitum. La Voie et al. (1981) indicated that nonsuckled cows had a shorter interval from parturition to first observed estrus ($P < .05$) than cows suckled twice daily or ad libitum. Suckling intensity also may be responsible for the delay in cyclic activity (Wyatt et al., 1977; Wettemann et al., 1978).

Nursing may affect the basal LH concentrations. Radford et al. (1978) reported that suckled beef cows had lower LH concentrations than nonlactating cows up to 30 days postpartum, with no differences thereafter. Beef cows suckled once a day had higher ($P < .01$) mean serum LH concentration than cows suckled twice a day (Forrest et al., 1980a). Milked Friesian cows developed episodic patterns of plasma LH between days 10 and 20 postpartum while few episodic releases of LH were seen in Friesian cows suckled by several calves (Lamming et al., 1981). Randel et al. (1976) also observed that suckling depressed the LH concentrations in beef cows. Nursing may affect LH concentrations by decreasing the frequency and magnitude of episodic release of basal LH (Carruthers and Hafs, 1980; Carruthers et al., 1980; Peters et al., 1981; Walters et al., 1982b; c). Eckternkamp (1978) suggested that suckling may suppress LH secretion by retarding the transition of the bovine pituitary from a state of low LH content during gestation to a state of higher LH content in cyclic cows. However, Carruthers et al. (1980) reported that cows suckled by two calves and also machine milked had the same pituitary concentration and total gland content of LH, FSH and prolactin compared to cows milked twice daily. The authors also reported that suckling did not affect serum concentration of P_4, E_2, prolactin, total glucocorticoids or FSH during the first 2 weeks postpartum. Hypothalamic concentrations of LHRH also were not affected by suckling, suggesting that this probably would not limit resumption of LH secretion in postpartum cows.

Carter et al. (1980) pointed out that reestablishment of reproductive activity after parturition is delayed by suckling through suppression of pituitary gonadotropin release, and this in turn delays ovarian follicular development. Removal of the suckling stimulus on day 21 postpartum led to an increase in pulsatile LH release, an in vitro increase in pituitary responsiveness to GnRH, an increase in basal serum LH and FSH concentrations, and an increase in LH and FSH receptors of the largest follicles (Walters et al., 1982a; b). Walters et al. (1982b) also observed that weaning had no effect on pituitary LH and FSH concentrations.

The milking machine stimuli do not appear to have an acute effect on the episodic release of LH in cows 10 to 14 days postpartum or 3 days following the first estrus postpartum (Rahe et al., 1980). Convey et al. (1983) also reported that suckling in beef cows had no acute effect on LH concentrations. Carruthers and Hafs (1980) observed that suckling increased the interval from parturition to first ovulation, depressed mean serum LH concentration and retarded the onset of episodic LH release.

Calf removal at 30 days postpartum induced a pulsatile secretion of LH in cows, while prolactin decreased to a basal concentration. This would indicate that prolactin should be reduced for the LH releasing mechanisms to be activated (Amoss et al., 1980). However, Gimenez et al. (1980) pointed out that prolactin had no inhibitory effect on posptartum anestrous due to suckling. Edgerton and Hafs (1973) reported that Holstein cows had higher prolactin levels at 7 to 8 weeks postpartum than during the first 6 weeks postpartum, but prolactin did not differ between anestrous animals, cycling postpartum animals or pregnant animals. Neither did prolactin vary significantly during the estrous cycle. Glucocorticoid concentrations, like prolactin, did not differ significantly between postpartum cycles. Lamming et al. (1981) observed that prolactin concentration declined linearly up to day 25 in suckled Friesian cows, while those in the milked group showed a gradual increase over the same period. Thereafter, up to day 90 postpartum, prolactin concentrations remained higher ($P < .01$) in the milked cows (27.1 vs 10.1 ng/ml) than in suckled cows. No correlations were found between prolactin concentrations and restoration of ovarian cyclicity. The higher prolactin concentration in milked cows suggested that hyperprolactinemia was not a cause of anestrous in postpartum cows, although suckling delayed the resumption of ovarian activity. Concentration of serum corticoids increases during milking (Smith et al., 1972) and suckling (Wagner and Oxenreider, 1972; Dunlap et al., 1981; Ellicott et al., 1981). Convey et al. (1983), using beef cows accustomed to handling, observed no effect of suckling on glucocorticoid concentrations in normally-suckled, intact cows, but, in ovariectomized suckled cows, suckling induced an increase in glucocorticoids.

Hormonal response (LH) to GnRH or estrogens may be influenced by the lactational status of the animal (suckled, milked, or nonlactating). Short et al. (1979) reported that suckling had no effect on the ability of suckled cows to release LH in response to injection of 10 to 20 mg of E_2. Injection of 20 mg of E_2 at 21 days postpartum induced LH surges in all suckled and nonsuckled cows, but in suckled cows the LH surge was shorter in duration than in nonsuckled cows. The LH response to either 0.25, 1 or 4 mg of E_2 was not reestablished before 2 weeks postpartum in suckled cows, but 10 mg of E_2 induced LH release either in suckled or ovariectomized cows prior to 2 weeks postpartum (Short et al., 1979). A significant inhibition of the postive feedback to estradiol benzoate (5 mg, i.m.), given at 28 h after an injection of cloprostenol, occurred during the early postpartum period (0-20 days). However, by the third week postpartum most animals responded to estradiol benzoate with both an

LH surge and estrus being expressed (Nancarrow et al., 1977). Foote (1971) observed that the ovaries during the early postpartum period in cows can influence gonadotropin production or release, since ovariectomy just after calving decreases the LH pituitary content by day 22 postpartum. This indicates that more LH is being secreted. Smith et al. (1983) reported that the basal LH concentration was higher in cows following calf removal for 24 or 32 h, than in suckled controls. They also observed that return of calves at the time of GnRH injection had a depressing effect on LH release.

In summary, suckling decreases mean basal LH concentration (Randel et al., 1976; Radford et al., 1978; Forrest et al., 1982) probably by decreasing the frequency and magnitude of episodic release of basal LH (Carruthers and Hafs, 1980; Carruthers et al., 1980; Peters et al., 1981; Walters et al., 1982b; c). However, the pituitary content of LH, FSH and prolactin is unchanged as is the hypothalamic concentration of LHRH (Carruthers et al., 1980; Walters et al., 1982b). Convey et al. (1983) observed no acute inhibitory effect of suckling on LH and FSH, confirming the observation of Rahe et al. (1980) that milking machine stimuli do not appear to have an acute effect on the release of LH. However, weaning causes changes in the episodic pattern of LH after 24 h (Walters et al., 1982a; b; Smith et al., 1983) indicating that suckling should be avoided for approximately 24 h so that its depressing effect on LH can be abolished. Although prolactin concentration increases due to suckling or milking stimuli, it does not delay the return to normal cyclicity in postpartum cows (Edgerton and Hafs, 1973; Gimenez et al., 1980; Lamming et al., 1981; Convey et al., 1983). Glucocorticoid blood concentrations are related to stress situations as are prolactin concentrations. It has been shown in an in vitro study that cortisol, in concentrations similar to circulating concentrations, inhibitied LHRH-induced LH release from bovine pituitary cells (Padmanabhan et al., 1983). However, Convey et al. (1983) demonstrated that glucocorticoids were not released by suckling in beef cows conditioned to handling but were released by suckled ovariectomized cows. The effect of ovariectomy was not understood.

Initiation of postpartum ovarian cyclic activity in cattle, thus, seems to depend on a "factor" that facilitates the increase in the concentrations of circulating LH in a pulsatile manner. The "factor" seems to be related to suckling, since suckled cows take longer to return to normal ovarian cyclicity postpartum than milked or nonlactating cows. Removal of the calf caused an increase in release of LH in a pulsatile manner which is similar to the normal hormonal changes (LH) observed before ovarian cyclic activity is restored during the postpartum period, (i.e., an increase in frequency and amplitude which seems to stimulate follicular growth and estrogen production). An increased concentration of estrogen can then exert a positive feedback on the hypothalamus causing a preovulatory surge of LH responsible for ovulation and (or) luteinization of the follicles. Luteinized follicles are rare and the first postpartum CL generally is difficult to palpate. These are the two structures that have been shown to produce enough P_4 to cause the first rise in P_4 during the postpartum period.

The suggestion that suckling increases the sensitivity of the hypothalamus to the negative effect of estrogen (Acosta et al., 1983) is attractive because

1. ovarian hormone(s) seems to have a depressing effect on LH concentrations since ovariectomy is followed by an increase in LH secretion.
2. injection of estrogen in ovariectomized cows reduces the LH concentration for up to 12 h, which seems not to be the case in intact cows. This suppressing effect of estrogen appears to be at the hypothalamic level since the pulsatile pattern of LH secretion is abolished after estrogen injection indicating that LHRH is not released in a pulsatile manner or is depressed to concentrations below the threshold of pituitary sensitivity.
3. implant of estrogen in ovariectomized suckled cows caused a greater reduction of LH concentration than implant of estrogen in ovariectomized early weaned cows.

Energy Source for Cattle

In simple-stomach animals, dietary carbohydrates are digested to glucose and other simple sugars, absorbed into the portal blood, and act as sources of glucose. In ruminants, most of the dietary carbohydrates are degraded anaerobically by the rumen microflora to VFA. Only a small amount of glucose usually is absorbed, and gluconeogenesis is of prime importance for the ruminant pattern of metabolism. Therefore, the supply of glucose precursors and the organs that synthesize glucose could be limiting factors for the overall performance of ruminants and even for their survival (Bergman, 1973). Bergman (1973) also indicates that glucose is needed by at least five systems of the body: 1, nervous system; 2, adipose tissue for turnover and synthesis of fat; 3, muscle; 4, fetus; and 5, the mammary gland. Cells of the nervous system, brain in particular, are absolutely dependent upon a regular supply of glucose for oxidative metabolism. The main substrates for gluconeogenesis in ruminants according to Bergman (1973) are 1, propionate; 2, glycerol; 3, amino acids; and 4, lactate and pyruvate. The conversion of propionate into glucose may undergo the following outlined reactions, as reviewed by Leng et al. (1967).

1a - Propionate $\xrightarrow{+CO_2}$ succinate $\xrightarrow{-CO_2}$ lactate

1b - Lactate $\xrightarrow{+CO_2}$ oxaloacetate $\leftrightarrows$ succinate $\xrightarrow{-CO_2}$ phosphoenolpyruvate $\rightarrow$ glucose

2 - Propionate $\xrightarrow{+CO_2}$ succinate $\xrightarrow{-CO_2}$ phosphoenolpyruvate glucose.

Reaction 1a occurs in the rumen epithelium and reactions 1a, 1b and 2 occur in the liver. The hepatic production of glucose accounts for the majority of total glucose produced. The mean rate of hepatic glucose production is about 85% of the total glucose turnover (Bergman, 1973). Production of glucose by kidneys accounted for 8-10% of the whole glucose turnover of the body.

It would be expected that an increase in propionate production would increase animal performance since increased propionate 1, will reduce loss of energy as methane and improve fermentation efficiency (Owens, 1980); 2, may lower the heat increment of the animal (Blaxter and Wainman, 1964); and 3, may spare amino acids normally used for gluconeogenesis (Leng et al., 1967; Reilly and Ford, 1971).

Monensin, an ionophore produced by a strain of Streptomyces cinamonensis (Haney and Hoehn, 1967), has been shown to improve feed efficiency and causes a change in the rumen acetate:propionate ratio. Richardson et al. (1976) observed from feeding monensin, that the molar proportion of propionate increased while the molar proportion of acetate and butyrate decreased. This theoretically would save 5.6% of energy for the animal. Rowe et al. (1981) concluded that when the diet was supplemented with monensin the availability of metabolizable energy (ME) increased by 20%.

Ionophores are lipophilic substances that bind ions and, due to their lipid solubility, facilitate diffusion of ions through lipid barriers (Austic and Smith, 1980). In prokaryotic cells, Bergen and Bates (in press) concluded in their review that the underlying mode of action of ionophores is on transmembrane ion fluxes and dissipation of cation and proton gradients. This action destroys primary membrane transport systems of cells, thereby interfering with cellular solute uptake that is coupled to primary transport systems. Cells respond to this metabolic change by maintaining primary transport by expending metabolic energy. Cells dependent on substrate level phosphorylation for adenosine triphosphate (ATP) can not meet this demand and lyse. However, cells capable of at least some electron transport coupled for proton extrusion and (or) ATP synthesis will exhibit a higher maintenance energy requirement but continue growth and (or) survive. Gram positive bacteria depend on substrate level phosphorylation and are inhibited by ionophores while gram negative organisms, many of which contain fumarate reductase, survive in the presence of ionophores. Thus, the gram negative population in the rumen is enriched.

In tissues, ionophores carry bound ions across membranes in directions favored by their concentration gradients (Austic and Smith, 1980). Monensin has a preference for sodium ions, i.e., it facilitates sodium entry into animal cells, and alters the activity of the sodium-potassium pump in the pericytoplasmic membrane. The increase in intracellular Na probably accounts for the stimulation of the pump by the ionophore for which the predominant effect clearly is to elevate cell Na and promote K exodus. In eukaryotic cytoplasmic membranes, the sodium-potassium adenosine triphosphatase (Na/K ATPase) is the primary transport system. This enzyme plays a central role in many different cellular functions including osmoregulation, excitation and solute transport. The Na/K ATPase establishes a Na gradient through expenditure of ATP and causes hyperpolarization of the cell membrane, because the stoichiometry of cation translocation is three ions of Na for two ions of K. Any membrane process or uptake system based on an electrical gradient, excitability or osmoregulation may be modified by polyether ionophores if effective levels get to the specific tissues (Bergen and Bates, in press).

Experiments with rabbits fed monensin (10 mg/kg) showed that monensin remains in the tissues after it clears the blood, and highest concentrations were observed in the liver, kidney, brain, heart. Low concentrations were found in fat and muscle (Fahim and Pressman, 1981). This tissue distribution suggests that ionophores seek out cell types of intermediate polarity, not predominately protein or neutral lipid. A candidate for such a tissue component would be the phospholipid fraction whose pool would be larger in metabolitically active organs. In rabbits fed monensin, it was observed that the rate of monensin absorption remained higher than its rate of elimination. Keeping in mind that monensin is highly soluble in lipids and that most of the injected monensin clears rapidly from the blood, one might expect that monensin when fed, would pass easily through the gut into the blood and be taken up readily by the tissue (Fahim and Pressman, 1981). It was evident also that the maximal permitted residue concentration of monensin in tissues of cattle and chicken set by the Food and Drug Administration, 0.05 ppm, would be very easily achieved and exceeded in even casual, nonchronic, feeding experiments. Herberg et al. (1978) pointed out that liver tissue from a steer conditioned for 2 days on ^{14}C-monensin and sacrificed 12 h after the last dose, contained 0.59 ppm of monensin (radioactive equivalent). When one heifer and two steers were conditioned for 5 days on ^{14}C-monensin and slaughtered 12 h after the last dose, the liver contained 0.214, 0.425 and 0.357 ppm of monensin (radioactive equivalents), respectively. Other tissues, such as round, kidney fat, subcutaneous fat, kidney and heart had less than 0.021 ppm. Donoho et al. (1978) observed that monensin was converted to a large number of metabolites by a steer and a rat. Biliary excretion in the steer appeared to be the primary excretory pathway for monensin metabolites. No direct measurements on the absorption of monensin were made. They also reported that monensin was not extensively degraded in the alimentary canal of the ruminant.

Fahim and Pressman (1981) indicated that, with the exception of a coronary vasodilatory response, all other hemodynamic effects of monensin are mediated in part by release of endogenous catecholamines. This was suggested because adrenergic antagonists or pretreatment with reserpine alter the hemodynamic effects. Infusion of monensin into the circulation of dogs was followed by an acute increase in blood glucose at 3 min which peaked by 90 min after infusion. In dogs and rabbits fed monensin, glucose levels peaked at 1.5 and 5 h, respectively (Fahim and Pressman, 1981).

Saini et al. (1979) suggested that injection of monensin in dogs releases both norepinephrine from the adrenergic nerve terminals and epinephrine from the adrenal medulla. This was suggested since the cardiovascular actions of a large dose of monensin were similar to those reported for both norepinephrine and epinephrine treatment.

Nutritional Effects on Reprodutive Performance

Wiltbank (1973) fed heifers different levels of total digestive nutrients (TDN) before (1.8 vs 3.6 kg of TDN) and after (3.2 vs 5.9 vs 10.5 kg of TDN) parturition and reported a 90% pregnancy rate in the low-high group (1.8 kg and 10.5 kg of TDN) and 70, 64 and 73% pregnancy rates in moderate-moderate (3.6 kg and 5.9 kg of TDN), moderate-low (3.6 kg and 3.2 kg of TDN) and low-moderate (1.8 kg and 5.9 kg of TDN) groups, respectively. When cows were fed high levels of energy, a greater number of cows were bred early in the breeding season along with a greater conception rate at these earlier services. A limitation of TDN to 50% of NRC recommendations, before or after parturition, delayed the onset of postpartum estrus (Wiltbank et al., 1964). In another study, Hereford cows were fed two levels of energy prepartum, 4.15 kg of TDN (high) and 2.1 kg of TDN (low), and each group was subdivided postpartum to receive either 7.3 kg of TDN (high) or 3.6 kg of TDN (low). Pregnancy rates were 20, 95, 77 and 95% for cows fed low-low, low-high, high-low, and high-high levels, respectively (Wiltbank et al., 1962). The occurrence of first estrus postpartum was affected significantly by level of nutrition, and the mean intervals to estrus were 52, 65, 43, and 48 days postpartum, for low-low, low-high, high-low, and high-high, respectively. Dunn et al. (1964) fed two levels of TDN prepartum (3.7 and 2 kg). During the postpartum period, the cows that received 3.7 kg of TDN prepartum were subdivided to be fed 10.3, 6 or 3.1 kg of TDN, and cows that received 2 kg of TDN prepartum were fed either 10.3 or 6 kg of TDN. Pregnancy rates were 81, 90, 70, 64 and 73% for groups receiving 3.7-10.3 kg of TDN, 2-10.4 kg of TDN, 3.7-6 kg of TDN, 3.7-3.1 kg of TDN and 2-6 kg of TDN, respectively. The intervals from parturition to first estrus were 56, 63, 50, 44 and 64 days, respectively. Postpartum body weight changes of cows were 88.5, 119.7, 36.3, -24, 61.7 kg in the same order as above. Cows that lost weight had the lowest pregnancy rate (64%). Dunn et al. (1969), working with 2-year old cows, observed that pregnancy rate at 120 days postpartum was related to postpartum energy level. Cows fed 48.2 Mcal of digestible energy (DE)(high) or 27.3 Mcal of DE (moderate) or 14.2 Mcal of DE (low) postpartum had 87, 72 and 64% pregnancy rate, respectively. A long, postpartum anestrous period was the major cause of the low pregnancy rate.

Bellows and Short (1978) reported that cows fed high levels of TDN (6.3 or 6.4 kg) before parturition had a shorter postpartum interval to conception, that a greater number showed estrus before the breeding season, and tended to have a higher pregnancy rate than cows fed low levels of TDN (3.2 or 3.4 kg).

Kaiser (1975) suggested the possibility of an interaction between plane of nutrition and suckling. Dunn and Kaltenbach (1980) indicated that an interaction between nutrition and suckling was a possibility and quoted Clemente (1978), who showed that the intervals from calving to first observed estrus were 15, 42, 27 and 70 days for cows gaining weight before parturition and nonsuckled, gaining weight before parturition and suckled, losing weight before parturition and nonsuckled, and losing weight before parturition and suckled, respectively. This indicates that the nutritional condition before parturition affects the interval to first estrus postpartum independent of suckling stimuli (15 vs 27 day intervals). However, suckling stimuli on cows that lost weight before parturition, almost doubled the interval from parturition to first estrus compared to cows that gained weight prepartum and were not suckled (70 vs 42 day intervals). The various reports (Wiltbank et al., 1962; Dunn et al., 1964; Wiltbank et al., 1964; Dunn et al., 1969; Wiltbank, 1973; Bellows and Short, 1978) indicate that an increase in nutritional levels (mainly energy) pre- and postpartum is followed by an increase in pregnancy rate. The highest plane of nutrition pre- and postpartum has not resulted always in the highest pregnancy rate. Similar or higher pregnancy rates often can be obtained with an intermediate level of nutrition before parturition and an increased nutritional level during the postpartum period.

In trying to compare and summarize studies with low or high levels of nutrition, based on TDN, we can observe that for the prepartum period Wiltbank et al. (1962), Dunn et al. (1964), Wiltbank (1973) and Bellows and Short (1978) called 4.15, 3.7, 3.6 and 6.3 kg of TDN, respectively, a high level of nutrition. However, the variations among the same studies appear to be less for what was called low (TDN) levels of prepartum feeding (2.1, 2, 1.8 and 3.3 kg of TDN). During the postpartum period, high levels were 7.3, 10.3 and 10.5 kg of TDN Wiltbank et al. (1962), Dunn et al. (1964) and Wiltbank et al. (1973), respectively. Moderate or intermediate levels were similar between Wiltbank (1973; 5.9 kg of TDN) and Dunn et al. (1964; 6 kg of TDN) as well as low levels postpartum (3.2 and 3.1 kg of TDN, respectively).

Low levels of TDN pre- and postpartum in general result in a low pregnancy rate; whereas a low prepartum feeding level followed by a high or moderate level of TDN during the postpartum resulted in a satisfactory to very good pregnancy rate. High levels of TDN before parturition followed by a low level of TDN postpartum resulted in a lower pregnancy rate than a postpartum moderate or high level of TDN.

Dunn and Kaltenbach (1980) pointed out that cows should maintain or gain weight from 100 days prepartum to the first few days postpartum, and then gain weight after parturition so that a short calving interval can be achieved. A high percentage of cows that were in good condition at parturition had shown estrus by 60 days postpartum regardless of the body weight change pre- or postpartum. However, cows with moderate body condition at parturition were more affected by prepartum weight change than postpartum weight change. More cows that gained weight prepartum and had a moderate body condition at parturition came in heat by 60 days postpartum than cows that lost weight prior to parturition (72 vs 49%). The majority of the cows that were thin at parturition failed to show estrus by 80 days postpartum compared with cows in moderate or good body condition, 75 vs 10 and 0%, respectively (Dunn and Kaltenbach, 1980).

Blood Glucose Concentration and Reproductive Performance

The effect of nutrition on ruminant glucose concentrations has been studied by Howland et al. (1966) in ewes and Oxenreider and Wagner (1971) and Downie and Gelman (1976) in cows. All of these studies indicate that increasing energy level increased glucose concentration. However, Dunn et al. (1974) reported no differences in glucose concentrations in cows fed two different diets (3.6 kg of alfalfa cubes vs 2.6 kg of alfalfa cubes + 2.3 kg of ground barley). A significant negative correlation between plasma glucose concentration and postpartum interval to occurrence of a 10 mm follicle and ovulation was observed by Oxenreider and Wagner (1971). Downie and Gelman (1976) showed that plasma glucose concentrations had a significant rise before service in fertile cows and a significant decline in the infertile cows. Patil and Deshpande (1979) reported that cows that did not show heat up to 90 days postpartum had lower glucose concentrations at the sixth week postpartum than cows that showed heat at 60-65 day postpartum. Cows that had lower glucose concentrations also lost weight throughout the experimental period, whereas cows with higher concentrations of glucose lost weight up to 4 weeks postpartum and then started gaining weight. Their work is in agreement with Downie and Gelman (1976), in respect to a decrease in body weight and plasma glucose, but the later authors also observed that the decreases of both body weight and glucose concentrations were characteristic of infertile cows. However, an increase in plasma glucose when body weight was decreasing was a characteristic of fertile cows. Rowlands et al. (1980) pointed out that poor fertility in their experiment did not appear to be related to low blood glucose concentration measured weekly. They also pointed out that there was no change in fertility even after increases in energy intake and glucose concentrations.

McClure (1972) suggested that by depressing nervous system activity, hypoglycemia inhibits hypothalamic secretion of GnRH. He also observed that the addition of energy-rich concentrates to the rations of infertile hypoglycemic herds resulted in significant increases in both blood sugar concentration and fertility. Administration of insulin daily from days 17 to 20 of the estrous cycle either inhibited estrus or reduced pregnancy rate (McClure, 1968). Walker et al. (1980a) did not observe differences in glucose level due to supplementation of monensin to cows. Rutter et al. (1983) reported a difference in glucose concentration when they infused heifers with propionate via the abomassum. This observation agrees with findings of Bergman et al. (1966) that the percentage of glucose originating from propionate markedly increases as more propionate is absorbed.

Glucose concentration seems to be important in the reproductive process, because it is the major source of energy to the central nervous system. An inadequate supply of glucose to the brain may cause hormonal changes that would influence the normal reproductive process. McClure (1972) suggested that hypoglycemia may influence the hypothalamus which would decrease the release of GnRH. The release of LH and (or) FSH would be abnormal and follicular growth would not be appropriate and the animal would remain anestrous.

Hormone Concentrations

Luteinizing hormone

There are contradictory findings concerning the influence of nutritional levels on hormonal concentrations in cattle. Dunn et al. (1974) measured LH concentrations in mature nonsuckled Hereford cows fed a restricted diet (3.6 kg of alfalfa) and a control group receiving 3.6 kg of alfalfa plus 2.3 kg of barley for almost two estrous cycles. Restricted diet cows had a higher peak of LH than the control cows (36.8 vs 23.8 ng/ml), although not significantly different. Concentrations of LH measured weekly did not differ between cows fed 130% or 70% of NRC requirements for TDN (Staigmiller et al., 1979). Hill et al. (1970) reported that underfed heifers from day 5 of the cycle to 3 to 18 days after mating had the same LH concentrations as did control heifers. Level of nutrition had no significant effect, either between nutrition groups or among successive estrous cycles, on systemic blood concentrations of LH over two and one-half estrous cycles. Other authors observed differences in blood concentrations of LH as a result of different nutritional levels. The majority of other workers, however, observed that by decreasing the nutrition level there was a decrease in the basal concentration of LH in the circulation. Jordan and Swanson (1979) reported that LH was lower ($P < .02$) in serum of cows fed 12.7% crude protein as compared to those fed 16.3 and 19.3% CP. Gombe and Hansel (1973) found that reduced energy intake in cycling heifers increased LH concentration, but Apgar et al. (1975) feeding Holstein heifers 100 or 60% of the TDN allowances with protein intake approximately equal, observed that restricted fed heifers had lower LH concentrations during the last 10 days of the experiment. Gauthier et al. (1983) also reported significant decreases in LH and FSH concentrations at 5, 15 and 30 days postpartum in underfed lactating beef cows, and that the correlation (r = .58; P < .01; no. = 19) between mean daily weight gain from 0 to 45 days postpartum and LH concentration at day 30 was significant. Echternkamp et al. (1982) also observed a high correlation (r = .75) between average daily weight gain and basal LH concentration of suckled beef heifers. Suckled beef heifers fed 100% of NRC recommended energy had a higher (P < .01) basal LH concentrations than heifers fed 60% of the recommendation. Underfed ewes also had lower basal LH concentrations than controls (Haresign, 1981).

Level of nutrition may influence the LH response to GnRH or estrogen. Beal et al. (1978) reported that nonsuckled beef heifers individually fed a low energy ration (3.9 Mcal of net energy per day) lost .28 kg per day and had higher peak concentrations of LH and a greater total LH release following the first of a series of five GnRH injections given at 0, 4, 8, 12 and 16 h, than heifers given 9 Mcal of net energy per day, which gained .33 kg per day. However, in nonsuckled beef cows, level of energy (10.6 or 6.9 Mcal of net energy per day) had no effect on LH peak value or total LH release in response to the GnRH challenge. It was observed also that nonsuckled ovariectomized cows that were fed 10.6 or 6.8 Mcal of net energy per day differ in LH response to GnRH. Ovariectomized cows fed a low energy ration had a greater (P < .05) response to GnRH than ovariectomized cows fed at high energy ration. It was suggested that the ovaries were not involved, and that restriction of energy intake presumably acted directly on the pituitary to increase the responsiveness to GnRH. No basal LH was measured at the time of challenge, but a low level of energy may have decreased the LH concentration in the blood due to insufficient stimulation of the pituitary. This, consequently, could increase the releasable LH pool in the pituitary of ovariectomized cows fed low levels of energy. In comparing the response to GnRH between heifers and cows, the authors pointed out that similar weight changes may not affect mature cows and growing heifers equally. Heifers, having lost a greater percentage of their body weight while still in a growing phase, may have been more critically affected. Furthermore, a lower dose of GnRH was administered in heifers (125 ug) than in cows (250 ug), and the effects of energy restriction on pituitary responsiveness to GnRH may have been dose dependent.

Lishman et al. (1979) reported that primiparous beef cows fed 115% of NRC energy recommendations released more LH than cows fed 50% of NRC. Two thirds of the cows in each nutritional group were injected with FSH (either .25 or .5 mg/day) from day 30 to 33 postpartum and on day 34 postpartum 300 ug of GnRH was injected i.v. During the first 30 days postpartum cows in the low group lost 31.5 kg of body weight while cows in the high energy group lost 10.7 kg. Neither level of feeding nor FSH affected daily concentrations of E_2 prior to or 8 h after GnRH. Also, FSH injection did not alter basal concentrations of LH (.2 ng/ml) or the pattern of release or the maximum concentration of LH in response to GnRH. Cows that had follicles greater than 9 mm released more ($P < .01$) LH due to GnRH than cows with follicles less than 10 mm prior to GnRH injection. Underfed ewes challenged with 150 ug of LHRH on day 12 of the estrous cycle during three different cycles released more LH than ewes fed adequately. This difference was observed at all three times that the ewes were challenged, but only during the last period were the differences significant (Haresign, 1981). This indicates that reduced sensitivity to the preovulatory LH surge mechanism is unlikely to be the major determinant of impaired reproductive efficiency in underfed ewes, which release more LH than well fed ewes.

Entwistle and Oga (1977) reported that suckled beef cows fed to gain or lose weight did not differ in LH response to 125 ug of LHRH when injected 60 days postpartum, although there was a trend for cows in the high level of nutrition to release more LH. All cows were anestrous at 60 days postpartum ($P_4 < 1$ ng/ml). Cummins et al. (1975) were unable to detect an effect of plane of nutrition on LH release due to LHRH in postpartum cows. Suckled beef heifers fed low levels of energy released less LH ($P < .01$) in response to an injection of 10 mg of estradiol benzoate given at 14, 32, 50 and 74 days postpartum than heifers fed a high energy ration (Echternkamp et al., 1982). It was observed also that suckled heifers challenged at 14 days postpartum released less LH ($P < .05$) than when challenged at 32, 50 and 75 days postpartum.

Luteinizing hormone response to GnRH and estrogens in heifers and cows fed monensin has been studied. Prepuberal heifers fed monensin released more LH to a GnRH (Randel and Rhodes III, 1980) or estrogen challenge (Randel et al., 1982) than control heifers. Prepuberal heifers infused with propionate, via the abomassum, released more LH in response to GnRH than controls (Rutter et al., 1983), indicating that an increase in absorption of propionate with subsequent conversion into glucose may be responsible for a greater pituitary activity or sensitivity to GnRH.

Although the majority of research agrees that a decrease in nutritional level would be responsible for a lower basal concentration of LH, it still is controversial as to how it happens. However, the majority of the times, it seems that cows or heifers that are receiving low nutritional levels tend to release LH in greater or equal amounts than the animal fed higher levels, indicating that probably responsiveness of the pituitary is not limited due to a low nutritional level. If responsiveness is not decreased but basal LH is decreased, then perhaps the release of GnRH from the hypothalamus in underfed animals is decreased. Consequently, the pituitary is less stimulated and basal concentrations of LH are decreased. This also may be responsible for an increase in the releasable pool of LH in the pituitary to an exogenous challenge of GnRH. The work of Beal et al. (1978) with primiparous cows seems appropriate to demonstrate this phenomenon. Underfed nonsuckled beef cows released more LH after the first of a series of five GnRH injections given at 4-h intervals but did not differ at the other four subsequent injections of GnRH. This would indicate that after the first injection of GnRH, LH content in the pituitary would be similar between cows of both nutritional levels and subsequent LH responses to GnRH are comparable, indicating that sensitivity to GnRH is not a problem in underfed animals. The difference may reside at a higher level of central nervous system control, i.e., hypothalamus. Perhaps a decreased secretion of GnRH exists because the necessary nutrients are not supplied at the desired levels, e.g., glucose. The theory that nutritional effects in reproduction may be in part related to hypofunction of the hypothalamus is in part supported by the fact that underfed cows and prepuberal heifers release less LH in response to estrogen than controls (Echternkamp et al., 1982; Randel et al., 1982).

Progesterone

Apgar et al. (1975) reported that the CL of Holstein heifers fed 100% of TDN allowances were heavier than CL of restricted-fed heifers (60% of TDN), with no difference in concentration of P_4 in the CL. Plasma progesterone levels were the same for both groups. The CL of these heifers were incubated with different levels of LH and it was noted that the P_4 increase during incubation was greater in the control than in the restricted-fed heifers, particularly at the higher concentrations of LH. Reduced energy intake in cycling heifers was responsible for a lighter CL and lower plasma concentration of P_4, and CL content of P_4 (Gombe and Hansel, 1973). Beal et al. (1978) also observed that beef heifers fed a low energy level diet (3.9 Mcal of net energy per day) tended to have lower concentrations of serum P_4 but in beef cows no differences in P_4 concentrations were observed. It was noted also that the CL of the low-energy-fed cows weighed less without an affect on the CL P_4 content or concentration, which is in agreement with Apgar et al. (1975) and Gombe and Hansel (1973). Progesterone concentrations from day 5 to 11 of the estrous cycle were increased in monensin fed heifers challenged with porcine follicle stimulating hormone (FSH-P, 1 mg at 12 h intervals from day 16 of the estrous cycle until day 21 or until heifers showed estrus)(Harrison et al., 1982). Dunn et al. (1974) reported that cows fed a restricted diet had lighter CL ($P < .01$) and lower P_4

concentrations in the CL ($P < .01$) than controls. However, P_4 plasma concentrations were higher ($P < .05$) for restricted fed cows than controls. Although there is a general agreement that the CL of underfed females is lighter than the CL from control cows or heifers, there are contradictory results on circulating concentrations of P_4. Apgar et al. (1975) observed lighter CL and no difference in plasma P_4 concentration while Gombe and Hansel (1973) reported decreases in both CL weight and plasma P_4 concentrations with low nutritional levels. Dunn et al. (1974) also reported a decrease in CL weight due to low nutritional level, but plasma P_4 concentrations were higher than in control cows.

Ovarian activity

Beef cows fed 150% NRC recommendation of TDN had greater ovarian activity, larger ovaries and larger follicles than cows fed lower energy levels (Wiltbank et al., 1964). Level of nutrition affected the ovarian response to injections of pregnant mare serum gonadotropin (PMSG) in ewes (Allen and Lamming, 1961) and beef heifers (Lamond, 1970b), with the nutritional effects more pronounced in the degree of follicular development than in the ovulation rate. Howland et al. (1966) reported that ewes fed grain had more ($P < .01$) large follicles, more ($P < .01$) follicular fluid and more ($P < .01$) CL than ewes fed hay. They suggested that the higher ($P < .01$) blood glucose of the grain-fed ewes could provide a stimulus to the hypothalamic centers controlling secretion of gonadotropins. This would lead to an increased production of hormones and subsequent ovarian activity. Anterior pituitary gland weight was increased ($P < .01$) by grain feeding. Spitzer et al. (1978), feeding cycling beef heifers 100 and

75% of NRC recommendations, found no significant differences in number of follicles, follicular or luteal volume. However, there was a tendency for the CL of heifers fed the low level diet to be smaller.

Staigmiller et al. (1979) reported that cows fed 130% of NRC recommendations had fewer total ovarian structures ($P < .05$), smaller number of large follicles ($P < .01$) and structures equal or greater than 10 mm ($P < .01$) than did cows fed 70% of NRC, after two daily injections of FSH for 5 consecutive days. Beef cows fed either 115 or 50% NRC recommended energy requirements showed no differences on size of the largest follicle at 30 days postpartum although cows fed 50% NRC lost more weight ($P < .01$) than cows fed 115% NRC within the 30 day period (Lishman et al., 1979). Butler et al. (1981) working with high producing dairy cows observed that the greater the average energy deficit incurred postpartum the longer the interval from parturition to ovulation. Suckled beef heifers fed a high energy ration had a higher incidence of ovulation by 46 days postpartum than low energy fed heifers (Echternkamp et al., 1982). Increased postpartum ovarian activity in suckled heifers fed the high energy ration postpartum may have been directly or indirectly related to the increased amount of releasable LH and increased tonic LH secretion. It should be pointed out that in this later study it could not be determined if the earlier appearance and increased magnitude of the pulsatile LH releases in heifers fed the high energy ration hastened ovulation or were the result of the earlier ovulation. Various authors have shown that before the first ovulation there is an increase in frequency and magnitude of LH (Rawlings et al., 1980; Humphrey et al., 1983).

Bushmich et al. (1980) observed that prepuberal heifers fed monensin had larger ($P < .025$) ovaries than controls at day 15 of monensin feeding. After an injection of FSH-P, monensin heifers had more ovulation sites ($P < .01$), CL numbers ($P < .05$), follicular fluid ($P < .01$) and total ovarian weight ($P < .05$). Harrison et al. (1982) observed an increase in CL weight and CL number from day 5 to 11 of the estrous cycle in monensin fed heifers challenged with FSH-P from day 16 of the previous estrous cycle to day 21 or estrus.

An increase in nutrition in general is followed by an increase in ovarian activity. This probably happens due to an increase in gonadotropin secretion (Howland et al., 1966; Echternkamp et al., 1982). Ovarian sensitivity to gonadotropins also may be enhanced due to better nutrition (Lamond, 1970b).

Age of puberty for heifers fed monensin was decreased (Moseley et al., 1977; McCartor et al., 1979; Moseley et al., 1982) or not affected (Nix et al., 1980; Sprott et al., 1980; Baile et al., 1982) compared to control heifers.

The effect of monensin feeding on postpartum cows has been studied. Turner et al. (1977) divided mature pregnant beef cows into two treatments with three replications to be carried out after parturition. Control cows received meadow hay (8.4% CP) free choice and a supplement of .45 kg of barley daily. The cows in the experimental group received 200 mg of monensin per cow daily that was incorporated into the barley. The supplement was fed individually. Cows were weighed every 28 days and rumen fluid samples were taken after 50 days on the experiment. In trial one, spring-calving cows (1974) were divided into two groups and received the treatments mentioned above. From November to February 1975, the second part of trial one was conducted. These cows were then maintained on their respective treatment throughout the summer, except during calving season (March to April) when they were group fed. A second trial was conducted similar to trial one except that monensin was fed on a 6 day schedule rather than daily, and no ruminal fluid samples were taken. Also during the last 64 days, barley was reduced from .45 kg to .23 kg. The winter portion of the trial finished on January 20, 1976, after 85 days of treatment. The results of these two trials indicated that monensin treated cows gained .20 kg and .19 kg per day more ($P < .05$) than controls during trials one and two, respectively. Monensin treated cows consumed less ($P < .06$) hay per day during trial one but no difference was observed in trial two. During trial one, monensin treated cows showed postpartum estrus earlier (30 vs 42 days; $P < .05$) than control, with no difference in trial two (49 vs 48 days). This happened probably because before the trial one cows were thrifty but with no excess of fat and after receiving good feed during trial one cows were carrying more fat than desirable. In another experiment (Turner et al., 1980), 96 pregnant beef cows were allotted to three replications of four treatments (0, 50, 200 and 300 mg of monensin per head daily). Monensin was mixed with .45 kg of barley. Meadow hay (9.5% CP) was supplied ad libitum to control, and 95% of the amount supplied to the control group was fed to the 50 mg group. The groups receiving 200 and 300 mg of monensin received 90% of the hay supplied to the control group. Cows were weighed at 28-day intervals prior to parturition, within 1 day after parturition, at the end of experimental feeding (30 days postpartum), and at weaning.

Average daily weight gain did not differ among treatments, but the monensin groups consumed 7 to 10% less hay indicating an increase in feed efficiency due to monensin. Considering a 50 kg loss in weight due to parturition for all treatments, a loss in weight from parturition to 30 days postpartum was observed in all four groups. Weaning weights at 139 days postpartum were not different. Days to first postpartum estrus did not differ and pregnancy rates were 84, 81, 100 and 86% for the groups receiving 0, 50, 200 or 300 mg of monensin, respectively. Volatile fatty acid concentrations were affected by treatment with 200 and 300 mg but not with 50 or 0 mg of monensin. Total VFA concentration was lower ($P < .05$) in the 300 mg group than in 50 mg group, 36.1 vs 41.1 mmoles/l. Also, 41.1 mmoles/l total VFA in the 50 mg group was lower than the 47.7 mmoles/l in the control group ($P < .05$). The control group also differed (47.7 vs 48.7 mmoles/l; $P < .05$) from the 200 mg group.

A study of the effect of breed, creep feeding and monensin on subsequent reproductive performance and lactation of 32 primiparous heifers was carried out by Hixon et al. (1982a). Eight Angus and eight Hereford heifer calves were creep fed and the other 16 heifers calves were not. Ninety days before weaning one-half of each group (creep-fed and not creep-fed) were assigned with their dams to an alfalfa and smooth bromegrass pasture and supplemented with a ration containing 2.67 Mcal of ME/kg (± .738 kg of TDN/kg of ration; Mcal of ME was transformed to TDN using the formula TDN = ME [Mcal/kg] ÷ 3.6155; NRC, 1976). From 40 days before the breeding season (180 day postweaning) all heifers were fed 87% of the NRC requirement for a gain of .45 kg daily and one-half of each breed and creep-fed groups received 200 mg of monensin. After parturition, the first-calf heifers were maintained in the same group and individually fed while remaining in a dry lot. Energy was adjusted to 90% of the NRC requirement for suckled cows of average milk production based on their postpartum weight (447 kg, 4.6 kg of TDN). Monensin cows lost 20 kg of body weight while control lost 25.4 kg. The interval from parturition to first estrus was significantly reduced by feeding 200 mg of monensin (56 vs 69 days). It was observed that monensin-fed heifers gained more weight over the gestation period and were in slightly better body condition at calving than those heifers not receiving monensin. These observations may indicate that feeding monensin during the postpartum period had no effect on postpartum interval to first estrus, since it has been shown that cows in better body condition at parturition have a shorter interval to first postpartum estrus (Dunn and Kaltenbach, 1980).

The reproductive performance of beef cows (40 Angus and 40 Hereford), fed a supplement with or without monensin for 2 years, was studied by Hixon et al. (1982b). Cows were divided according to calving day to receive 85% of energy requirement (± 5 kg of TDN for the first year or ± 5.7 kg of TDN for the second year) for the initial period of 56 days. Cows in the first year were 56 days postpartum and weighed 455 kg. Cows in the second year were 46 days postpartum and weighed 472 kg. Monensin treated cows received 200 mg of monensin per head daily. Cows were group fed and calves were separated from the dams during the feeding time. After 56 days on trial cows were allowed to consume forage (corn silage) ad libitum for a 4 h period daily up to 140 days. After 30 days on feed, cows were synchronized with Synchro-Mate-B (Ceva).

Calves were separated from cows from the time of implant removal to 48 h after, at which time cows were artificially inseminated. Cows were rebred up to 90 days after the initial synchronized estrus. Blood was collected from 50% of each treatment group of cows at the time of implant removal and from 24 to 36 h after implant removal at 3 h intervals. These samples were assayed for LH concentration. For P_4 assay, blood was collected at implant removal, and at 24 h, 9 and 16 days after implant removal. Body weights were not affected by breed, treatment, or year. No differences were observed due to treatment or percentage of cows exhibiting an LH peak, time of peak or the value of the peak. Fewer monensin treated cows (45 vs 70%) had an LH peak within the bleeding time, compared to controls but the difference was not significant. Monensin did not affect P_4 concentrations. It was observed also that monensin supplementation did not result in a significant effect on calf weight or milk production at either 56 or 140 days of the experiment. Schalles and Fleck (1979) fed beef cows 0, 200 or 400 mg of monensin per head daily from November 1 to April 20 for two years. Cows were in a bluestem pasture year around and were supplemented with .74 kg of alfalfa hay and .45 kg of cube containing 70% sorghum grain, 25% soybean meal, and 5% molasses, per head daily from November 1 to February 15. From February 15 to April 20, an additional 2.72 kg of sorghum grain was fed. Cows fed monensin (200 mg) lost less weight ($P < .001$) from November to May (-36 vs -61 kg) than control animals or those fed 400 mg monensin (-72 kg). First service conception rate and final conception in a 56 day breeding season were 21% and 71%; 40% and 84%; 24% and 74% in the groups fed 0, 200 and 400 mg of monensin per head daily. Calf birth weights and calf gain from birth to weaning were not affected by treatment of their dams. In another experiment, Hereford cows (no. = 100) were fed 0, 50, 200 and 300 mg of monensin. Control cows received 100% of NRC recommendation of TDN while cows that received 50 mg of monensin received 95% of the TDN recommendation. Cows fed 200 or 300 mg of monensin received 90% of the TDN recommended by the NRC. With this design, Walker et al. (1980a) were able to maintain a similar weight change with few adjustments. They observed no differences in cow weight, condition score and heart girth changes for the prepartum period, calving and postpartum period, which finished at about 73 ± 13 day postpartum, and during a pasture period without monensin feeding. There was no difference among treatments on the interval from parturition to first insemination (means range from 67 to 74 days) or first service conception rate (mean 36%, means range from 31 to 56%). In another study, Walker et al. (1980b) fed beef cows 0 or 250 mg of monensin per head daily. Cows were synchronized between 44 and 80 days postpartum with Syncro-Mate-B plus 3 mg of norgestomet (i.m.) and 6 mg of estradiol valerate. Implants were removed 8 days later at which time the calves were separated from the cows for 48 h. Cows were inseminated 48 and 52 h after removal of implant. After insemination cows were put with fertile bulls. There was no difference in conception rate due to monensin or Syncro-Mate-B treatments.

Luteinizing hormone response to challenge with GnRH or estrogen in cows fed monensin has been studied. Hardin and Randel (1983) used 48 spring calving, Brangus cows in a 2x4 factorial design to study the effect of 0 or 200 mg of monensin and 0, 1, 2 or 4 mg of E_2 on LH response, interval from parturition to first estrus, and weight change. Cows were supplemented with a mixture of 2.27 kg of milo plus .45 kg of cottonseed meal. Coastal bermudagrass hay was offered ad libitum. Experimental cows received 200 mg of monensin in the concentrate. At 20 days postpartum, a controlled suckling regimen was initiated. Calves had access to their mothers for 2 h at 6 h intervals. On day 21, a single i.m. injection of 0, 1, 2 or 4 mg of E_2 in corn oil was administered. After E_2 injection, blood samples were collected for 48 h at 2 h intervals. Body weights at the time of challenge were similar. The interval from parturition to first estrus was shorter for monensin-fed cows (65 vs 86 days). Seventy-nine percent of monensin-fed cows had expressed estrus by 85 days postpartum compared to 50% in the control group. The LH response (ng/ml) to 0, 1 or 2 mg of E_2 was unaffected by diet. The interval from the injection of 4 mg of E_2 to the initiation of LH surge and to LH peak was longer ($P < .01$) for monensin than control cows.

LH response to GnRH in suckled beef cows supplemented with monensin was studied by Mason and Randel (1983). Brangus cows (no. = 27) were divided into nonsuckled monensin (NSM), suckled monensin (SM), nonsuckled control (NSC), and suckled control (SC) groups. Cows were fed 1.82 kg per head daily of a 3:1 mixture containing ground milo and cottonseed meal containing 0 or 200 mg of monensin. Costal bermudagrass hay was fed ad libitum. This would supply 100% of the recommended ME (± 5 kg of TDN). The conversion from ME to TDN for comparison was done by using the formula TDN = ME (Mcal/kg) ÷ 3.6155 considering the requirement of a beef cow of average milk production (NRC, 1976). Suckling treatment started at 0800 h on day 21 postpartum and lasted for 30 hours. Bleeding for LH lasted 6 h starting at 0800 h on day 22. The calves were allowed to suckle for 30 min at 6 h intervals in the SM and SC group. The NSM and NSC cows were separated from their calves from 0800 h on day 21 to 1400 h on day 22. An injection of 100 ug of GnRH (i.m.) was given 24 h after suckling treatment started. Cow weights did not differ by suckling or dietary treatment, but monensin treated cows had higher body condition scores ($P < .06$) at 21 days postpartum and at first estrus than controls. The interval from parturition to first estrus was shorter (92.4 vs 138 days; $P < .025$) for monensin than control cows. A greater proportion of monensin cows (8 of 14 vs 0 of 13; $P < .005$) had shown estrus by 90 days postpartum. All cows lost weight ($P < .075$) and body condition ($P < .005$) from day 21 postpartum to first estrus. Interval from GnRH to first estrus was not affected by treatment. It was observed that basal LH did not differ among treatments and that suckled cows released more LH in response to GnRH than did nonsuckled cows ($P < .005$). Cows fed monensin responded to GnRH with the release of less LH (P .005) than did cows not fed monensin.

The amounts of TDN fed or supplemented during the experiment with monensin were not always shown because the animals were not individually fed or were individually supplemented without control of hay or pasture consumption. For any comparison to be done, it would be necessary to have at least an estimated amount of TDN fed to the control cows. This estimation, when possible, indicated that cows were fed approximately 5 to 6 kg of TDN a day with this amount depending on weight and milking ability of the cow. A general conclusion would be that cows fed monensin tended to have a shorter interval from parturition to first estrus with no difference in pregnancy rate. Postpartum body weight change in the majority of research reports was not affected due to monensin feeding. Also, the effect of monensin treatment seems to be related to body condition of the animal (Turner et al. (1977). Heifers fed monensin attained puberty earlier than controls.

The restoration of ovarian cyclicity after parturition depends on a series of factors and (or) changes that must happen before the animal is able to conceive and maintain a normal gestation. Uterine and cervical involutions are rapid during the first 15 to 20 days followed by a more gradual decrease in size to be completed by 30-35 days postpartum as detected via rectal palpation and by 50 to 60 days postpartum as observed microscopically. Although gross uterine involution is not affected by nutritional level, suckling may enhance uterine involution. Hormonal changes also occur concomitantly with the involution of uterus and cervix.

A gradual increase in LH and P_4 occurs during the postpartum period. The LH increase is influenced negatively by suckling, which seems to act at the higher center(s) of the central nervous system. Suckled cows, in general, have a lower basal LH concentration and longer interval from parturition to first estrus than milked or nonlactating cows. This lower basal LH concentration (characterized by a decreased frequency and magnitude of episodic release) apparently does not stimulate sufficient follicular growth to produce estrogen in an amount capable of inducing the LH surge which precedes the first ovulation. The removal of suckling stimuli causes an increase in pulsatile LH release without affecting pituitary LH and FSH concentrations. The initiation of ovarian activity during the postpartum period is preceded by an increase in frequency and amplitude of LH release which culminates with a greater follicular growth. Estrogen production is responsible for the LH surge which is followed by ovulation or luteinization of follicles. These structures (which in the majority of cases are not palpable) are responsible for the first increase in P_4 after parturition which is, in general, of shorter duration than the normal 14 days.

Nutritional level is also a very important factor, if not the most important factor, affecting postpartum reproductive performance. It is known that level of nutrition before and (or) after parturition affects the calving interval, and to maintain an interpartum of 365 days, cows must become pregnant during the first 80 days postpartum. Cows that have a good body condition at parturition have a shorter interval to first estrus postpartum, assuming normal nutrition postpartum, than those that have a poor body condition. An increase in the level of nutrition during the postpartum period also decreases the interval to first estrus and to conception.

Various studies related to nutrition and hormonal response to GnRH or estrogen have been carried out. Responsivness of the pituitary of suckled cows to GnRH is restored by 2 weeks postpartum whereas restoration to estrogen induced LH surge seems to take longer than to GnRH induced LH surge. A majority of the studies reported that by decreasing the nutritional level there was a decrease in basal LH concentration. However, P_4 concentrations may or may not be affected by changes in levels of nutrition. The pituitary seems to release less LH but not due to a decreased responsiveness to LHRH, since the pituitary of underfed cows responds similarly to normal fed cows challenged with GnRH. So, the low basal LH concentration in underfed cows indicates that the pituitary is not being stimulated properly, probably due to a decrease in release of GnRH from the hypothalamus. A suboptimal supply of nutrients to the brain could have a depressing effect on brain impulses and (or) hormone production. A low concentration of glucose could decrease the secretion of gonadotropin as suggested by McClure (1972).

MATERIALS AND METHODS

Twenty-eight, 6 to 7-years-old pregnant, crossbred cows (9 Hereford x Angus, 3 Angus x Hereford, 10 Penzgauer x Angus, 4 Penzgauer x Hereford and 2 Brown Swiss x Angus) were used in the experiment. All the cows were bred to Brahman bulls. Animals were weighed and a condition score assigned according to Andrade (1980). The condition score scale varied from 1 to 17 where a condition of 1 was assigned to a very thin deformed cow, a condition of 8 to a cow with an estimated .20" to .25" of fat over the 12-13th ribs and a condition of 17 to a cow with more than 1.2" of fat over the 12-13th ribs. The experiment started 45 days before the start of the calving season which lasted from January 2 to February 14, 1983.

The cows were maintained on pastures of bahiagrass (IFN-2-00-464, NRC, 1982) and white clover (IFN-2-01-380), and supplemented as a group with an average of 3 kg of ground corn (IFN-4-26-023) and 6.8 kg of Costal Bermudagrass hay (IFN-1-00-716, 4.6% crude protein) per head daily for a period of 42 to 85 days prepartum. Four cows that had low condition scores (mean = 4.8, range 4 to 5) received an additional 1.5 kg of ground corn per head daily for 19 days. A mineral-salt block was supplied ad libitum to all cows throughout the experiment.

One cow lost her calf due to calving difficulty and another cow's calf died within 24 h of birth. Each of these cows then suckled a foster calf (Holstein male). One cow had a retained placenta and was treated with Furacin.

Cows and calves were weighed and a blood sample taken from the dam within 24 h after calving. The calves stayed with their mothers except during daily feeding and when the cows were bled every three days. All cows were fed individually a 16.6% crude protein supplement (74% corn, IFN-4-26-023 and 26% soybean meal, IFN-5-04-600, appendix table A-16) according to their metabolic weight. For the first 9 days postpartum, all cows received the same supplement. After this period, cows were stratified by day of calving and breed to two treatment groups (0 or 200 mg of monensin per head per day). All cows were fed the same concentrate mixture except that the monensin group had monensin added to it. Each cow in the monensin group was fed 200 mg of monensin daily. Monensin was mixed with the concentrate so that 1.5 kg of concentrate contained 200 mg of monensin. The amount of supplement fed per cow daily was adjusted at 12 days intervals if cow weight increased above first postpartum weight. If a cow lost weight after parturition, no adjustments were made in the amount of supplement fed.

Since total feed intake could not be controlled because cows were on pasture, an attempt was made to supply at least the minimum energy and protein requirements for beef cows of average milk production, according to their metabolic body weight (NRC, 1976). For a cow of average milk production that weighs 450 kg, the metabolic weight is 97.7 kg ($450^{.75}$) and the minimum requirement of dry matter, crude protein and total digestible nutrients are 9.3 kg, 0.86 kg and 5 kg, respectively. The total daily amount of supplement fed per cow was weighed daily. Nutrients (CP and TDN) requirement calculations are presented in appendix table A-26. One and one-half (1.5) kg of the supplement with monensin was weighed in plastic sacks identified for each cow and the total amount of concentrate required daily was adjusted by adding the control supplement. The average amount of concentrate supplemented daily per cow is presented in appendix table A-1. The Costal Bermudagrass hay consumption was considered fixed, and an average of 8.6 kg of hay was supplied daily per cow on pasture.

The second part of the experiment consisted of dividing each dietary group into two hormonal subgroups (n = 7) to receive injections of either 100 ug of GnRH in sterile solution (Gonadorelin, Abbott) or 10 mg of E_2 (Sigma) dissolved in 2 ml of sesame oil. This challenge occurred at 41 ± 1.5 days postpartum (Appendix table A-18). Therefore, responses after 41 days postpartum were evaluated in a 2x2 factorial design. The jugular veins of all cows were cannulated -1.5 to -0.5 h before blood sampling was started. The bleeding schedule for the GnRH group was every 20 min from -4 to 0 h. After the 0 h sample, 100 ug of GnRH in 2 ml of sterile solution was injected, via cannula, and flushed with 4 ml of sterile saline. After GnRH, blood samples were collected at 5 min and then at 10 min intervals from 10 to 120 min and at 30 min intervals from 120 to 360 min. Blood samples were put in an ice water bath or centrifuged immediately after sampling for 10 min at 2000 x g at room temperature. Plasma was stored at -15 C until analyzed for LH. The blood sampling schedule for cows treated with E_2 differed from GnRH cows during the period after 0 h. Just after 0 h, 10 mg of E_2 was injected intramuscularly, and cows were bled for 30 h at 1 h intervals. Blood samples were handled as in the GnRH group.

For both GnRH and E_2 treatments, cows were confined in individual stanchions in a shed with a concrete floor. Supplement, hay and water were supplied to the cows during the sampling period. Calves were allowed to stay with the cows throughout the bleeding period and nursed ad libitum. It appears that during the bleeding period the calves nursed more than usual, because they were confined.

Cows were supplemented with the experimental dietary treatment up to 90 days postpartum or until pregnancy was detected via nonsurgical embryo recovery at 17 to 18 days postbreeding. One cow was detected pregnant via rectal palpation, but only the data up to day 17 of pregnancy were analyzed. Cows were not bred before the hormone challenge.

Daily cow management consisted of herding the cows with a horse to the corral every morning and separating the calves from their dams which then were separated into the two feeding groups (monensin and control). Cows then were moved to individual stanchions where they received their respective supplement with or without monensin. Not all cows entered the stanchions during the first few days of the experiment, and these were fed individually in the corral. Cows were checked for estrus twice daily, am and pm, with two epididymectomized bulls with deviated penises wearing chin-ball markers. The bulls under anesthesia had their penises surgically modified by exposing the penis and making two tranverse incisions, 2 cm apart, on the dorsal ligament of the penis and the two 2 cm portions removed so that copulation could not occur. Cows in heat after the hormonal challenge and prior to 90 days postpartum were mated by a fertile bull.

Cows were bled via the jugular vein every third day before feeding the supplement. Blood was collected in vacuum tubes containing 80 IU of heparin, immediately put in an ice water bath, centrifuged at 4 C at 2000 x g, and the plasma stored at -15 C until analyzed for P_4, LH and glucose. Palpation per rectum was made every third day starting 10 days postpartum to determine uterine location and tonus, cervical and uterine horn diameters (mm) and ovarian dimensions (length, height, width in mm). Ovarian structures were palpated and their location and dimensions (mm) were estimated (Casida, 1960).

Uterine location was evaluated based on its position; a score of 0 was given if it was located in the abdominal cavity. A score of .5 represented a uterus half-way between abdominal cavity and pelvic cavity and a score of 1, represented an uterus in the pelvic cavity. Uterine tonus was considered to be normal, if tonus was typical of a cycling cow, and was assigned a score of 2, and if the consistency of the uterus was flacid without any tonus, a score of 1 was given.

Total ovarian volume (OV; cm^3) was calculated by multiplying the estimated ovarian dimensions of each ovary (length x height x width) and then adding the right and left ovarian volumes. Uterine size was calculated by adding the estimated diameter of each uterine horn measured close to the external bifurcation.

Scoring of ovarian structures was done by assigning a value of 1 to an ovary containing a CL or one or more follicles $\geq$ 6 mm in diameter. A value of 0 was given when no structure was detected. Total number of follicles or number of corpora lutea was determined by adding total follicles or CL present on the right and left ovaries.

A cumulative value was calculated by the addition of each measurement taken every 3 days postpartum. The cumulative value was used to minimize the within and among animal variation. This variation occurs because each cow is unique in starting its normal postpartum cyclicity; i.e., they are not synchronized. Each cow contributes differently to the average cumulative value which is represented by a single function. The rationale for using cumulative value, such as P_4 concentration, is that collectively occurrence of an increase in P_4, the number of times that P_4 increased, and all individual parameters describing P_4 increase contributed to the overall postpartum response of the animal for the period studied.

One sample of rumen fluid was taken 6 ± 0.5 days after the hormonal challenge. The sampling was done 6 to 8 h after the cows were fed supplement, by use of the suction strainer technique (Raun and Burroughs, 1962). The suction strainer technique was demonstrated to be similar to rumen fistula sampling for evaluation of molar proportion of rumen VFA in sheep (Beede et al., 1978) and in steers (Beede et al., 1975). The rumen fluid sample of about 70 ml was put in a plastic vial containing 3 ± .3 g of metaphosphoric acid, the vial shaken, the gas allowed to escape, sample cooled down and then frozen. Analyses of propionate, acetate, butyrate and total volatile fatty acid concentrations were done by Lilly Research Laboratories, Greenfield, Indiana.

Luteinizing Hormone Assay

Plasma concentrations of LH were measured utilizing modifications of the procedure described by Niswender et al. (1969). Unknown samples (100, 200 or 300 ul) were added to borosilicate tubes. Volumes were brought to 500 ul with 1% bovine serum albumin (BSA) in phosphate buffer (1% BSA-PB, .05 M, pH 7.4), (appendix test 1). Standard displacement curves were calculated from triplicate samples of standard LH (USDA-bLH-B-5; gift from USDA Animal Hormone Program and National Hormone and Pituitary Program) at concentrations of .06, .12, .25, .5, 1, 2.5 and 5 ng per tube in 1% BSA-PB, .05 M, pH 7.4. Standard tubes were brought to a volume of 500 ul utilizing 1% BSA-PB, .05 M pH 7.4. Two hundred microliters of rabbit anti-bovine LH (AB-B-225; gift from G.D. Niswender), diluted to 1:140,000 with normal rabbit serum (NRS; 1:400 dilution in EDTA-PB, pH 7.0, appendix test 1), were added to each tube except total count (TC) and non-specific binding (NSB) tubes. All tubes were incubated for 24 h at 4 C, and then 50,000 cpm of ^{125}I-LH per 100 ul of 1% BSA-PB, .05 M, pH 7.4 were added to each tube and incubated for 24 h at 4 C. The second antibody (sheep antirabbit-guinea pig gamma globulin serum-SARGPGG; gift from Dr. J.H. Britt, North Carolina State University), diluted 1:30 in NRS-EDTA-PBS, pH 7.0, was added to a 6% polyethylene glycol (PEG-6000) solution in PB, .05 M, pH 7.4 (appendix test 1) at a volume ratio of 20 ml of second antibody solution to 50 ml of 6% PEG-6000 solution (appendix test 1). Second antibody PEG-6000 solution (700 ul) was added to each tube, except TC tubes, the tube shaken, incubated for 15 min at room temperature and tubes centrifuged for 30 min at 4 C and 3000 x g. Supernatant was decanted, tubes inverted and pellets allowed to dry for approximately 30 min. Tubes with pellets were counted in a gamma counter (Tracor Analytic-Model Gamma-Trac, no. 1191) for 30 seconds each. Different volumes (25, 50, 100, 200 and

300 ul) of a plasma sample known to have a high concentration of LH (22 ng/ml) were assayed. The linear regression (R^2 = .98) for the logit plot of the displacement curve (Y = 9.2065578 - 2.39404807X; Y = ln of B/F, X = $\log_{10}$ of assay volume) was parallel to the displacement curve for the standard curve (Y = 11.709634 - 2.1713181X where Y = ln B/F, X = $\log_{10}$ of standard LH concentration, appendix figure A-1). Addition of .25, .5, 1, 2, 4, 6, 8, 10 and 12 ng of USDA-bLH-B-5 to 1 ml of cow plasma gave a linear recovery of LH (Y = .26620545 + [1.03020066x] when assaying 100 ul of plasma and Y = .37324948 + [0.88116202x] when assaying 200 ul of plasma where Y = ng/ml of hormone recovered and x = ng/ml of hormone added; appendix figure A-2). The intercept values closely approximated the LH concentration in the cow plasma of .3 ng/ml. A test for homogeneity of regression indicated that the two lines were not parallel.

The estimated value for added mass given by the regression for 100 ul assay volume is essentially ideal with a slope of 1.03 (a slope of 1.00 is ideal). However, the regression determined from the 200 ul assay volumes underestimated, based on the slope of .88, high concentrations in the plasma mainly those above 6 ng/ml. For example, 8, 10 and 12 ng/ml of standard LH added to plasma had a recovery of 7.4, 9.1 and 10.9 ng/ml, respectively, when assaying 200 ul of plasma. The 100 and 200 ul assay regressions agreed closely when plasma concentrations of LH were below 2 ng/ml (appendix figure A-2).

All samples assayed were either 100 or 200 ul; therefore, the estimates were valid in samples following GnRH (mean peak value = 12 ng/ml) which were accurately measured in 100 ul of assay volume. The underestimation of LH in 200 ul assay volume at high concentrations of LH appears to be due to some interactions between radioactive LH, endogenous LH and plasma protein with PEG and distribution of free radiolabelled LH. The anti-bovine LH (AB-B-225) serum dilution bound 23 to 32% of added ^{125}I-LH. The intra- and interassay coefficients of variation were 11.96% and 17.09% for a plasma sample from an ovariectomized cow ($\overline{X}$ = 0.76 ng/ml), and 6.89% and 9.32% for a sample of plasma, from ovariectomized cows containing 2 ng of exogenous LH per ml ($\overline{X}$ = 2.77 ng/ml).

Progesterone Assay

Progesterone concentrations were measured utilizing the method described by D. Wolfenson et al. (submitted). After extraction of P_4 from the plasma sample (200 ul), by utilizing 2 ml of benzene:hexane (1:2 ratio) in 16 x 100 mm tubes and vortexing for 30 seconds, samples were frozen at -15 C, and solvent transferred to assay vials (12 x 54 mm). Solvent was dried under air flow in a water bath at 37 C. Mean recovery of preg-4-ene-3,20-dione-1-2-^3H (^3H-P_4; 3,000 CPM per vial) was 89.3%. Standard curves were run in triplicate with P_4 concentrations of 31.25, 62.5, 125, 250, 500, 1000 and 1500 pg per 100 ul of PBS plus gelatin, pH 7.5 (PBSG; sodium phosphate monobasic - $NaH_2PO_4H_2O$ - 119.59g; sodium phosphate dibasic [Na_2HPO_4] - 40.175 g; sodium azide - 8.5 g; sodium chloride - [NaCl] - 21.5g; gelatin - 8.5 g; deionized water 8.5 l). Two milliliters of benzene-hexane solution were added to each standard curve tube and dried. One hundred microliters of standard were added to each of the tubes in triplicate. To unknown and reference samples as well as nonspecific binding tubes, 100 ul of PBSG were added to each tube. Four hundred microliters of a solution of rabbit γ globulin (20 mg/l) in PBSG were added to each tube. Then 100 ul of progesterone antibody (1:7,000) and 20,000 CPM of ^{3}H-P$_4$ in 100 ul of PBSG were added. Progesterone antiserum (PR-2 no. 24; donated by J.L. Fleeger of Texas A&M University) was produced in a rabbit against 11 -hydroxyprogesterone hemisuccinate conjugated to bovine serum albumin, and with specificity described by Eley et al. (1981). The specific activity of ^{3}H-P$_4$ was 50.3 ci/mmole (counting efficiency was 62%). Tubes were incubated for 30 min in a water bath at 37 C, and then 50 ul of a undiluted second antibody (sheep anti-rabbit serum) were added to all tubes except total count tubes. After an incubation for 12 h at 4 C, 2 ml of PBSG were added, tubes centrifuged for 30 min at 3000 x g (except total count tubes), and supernatants poured off. Ethanol (300 ml) was added to each tube (except total count tubes), all tubes were vortexed, and 2.5 ml of toluene-cocktail (14.96 g of 2,5 diphenyl oxazole [PPD, FW-221.25] and .2 g of 1.4 Bis [2-(4-methyl-5-phenyl oxazoyl)] benzene [POPO, FW-392.44] added to 4 l of toluene) added to each tube except total counts to which 2.5 ml of triton cocktail (800 ml of toluene-cocktail plus 200 ml of Triton-X [Fisher]) were added. The intraassay coefficient of variation was 30.4%.

Glucose Assay

Plasma glucose concentrations were determined by an enzymatic colorimetric method on deproteinized plasma (Sigma Tech. Bulletin. no. 510, 1982). Deionized water (dH$_2$0; 1.8 ml) was added to 12 x 75 mm flint tubes followed by 200 ul of the plasma sample in duplicate. For the standard curve tubes (25, 50, 75 and 100 mg/dl; duplicates), 200 ul of each dilution were added to 1.8 ml of dH$_2$0. The blank tubes received 2 ml of distilled H_2O. A control plasma sample was assayed in duplicate in each assay of unknown samples. To all the tubes, 1 ml of barium hydroxide solution ($Ba[OH]_2$-0.3N) was added, vortexed and then 1 ml of zinc sulfate solution ($ZnSO_4 \cdot 7H_2O$, 5%) was added. The tubes were shaken individually by hand followed by centrifugation (3,000 x g) at 4 C for 15 min. Supernatant was transferred to 12 x 75 nm flint tubes and stored at 4 C overnight. Four hundred microliters of the supernatant were transferred to 13 x 100 mm flint tubes in duplicate, 4 ml of the combined enzyme color reagent solution added, vortexed and incubated at room temperature for 45 min in a light protected place. The absorbance was read in glass cuvets at 450 nm in a double beam grating spectrophotometer (Beckman DB-G). Plasma glucose concentrations (mg/dl) were calculated using the formula, absorbance of the sample divided by absorbance of the standard (100 mg/dl) and multiplied by 100, where 100 represents the concentration of the glucose standard in mg/dl. The inter- and intraassay coefficient of variation were 0.42 and 0.52%, respectively.

Statistical Analyses

Hormone concentrations (P_4 and LH), glucose concentrations, number of follicles (NFO), number of CL (NCL), ovarian volume (OV), uterine size (UTSIZE), uterine location (UTLO), uterine tonus (UTONUS), diameter of the cervix (CVX) and postpartum cumulative responses of P_4 (CP4), LH measured every 3 days (CLH), glucose (CGL), number of follicles (CFO), number of CL (CCL) and ovarian volume (COV) were analyzed by the method of least squares analysis of variance (SAS, 1979). Polynomial response curves were used to describe time trends. Test of homogeneity of regression (Snedecor and Cochran, 1980) was done to detect differences among treatment response curves. Different mathematical models were used in the analyses. Time (days and hours), unless specified, was used as a continuous independent variable. The general model utilized for postpartum measurements (except for UTLO and CVX) during experimental periods up to 90 or 77 days postpartum or after hormonal challenge, included the following effects: supplement (S), hormone (H), the SxH interaction (SxH), cow within SxH [Cow(SxH)] and coded day (D). Day postpartum was coded as follows: 0.66666 + (d x 0.33334) = D, where d is the uncoded day postpartum for the analyses up to 38, 77 or 90 days postpartum. For the measurements analyzed before hormonal challenge, the mathematical model included effects of S, cow within S [Cow(S)] and D. The mathematical model for LH response due to hormonal challenge (GnRH or E_2) consisted of S, Cow(S) and hour (Hr).

The variance of basal LH concentrations before challenge was tested by comparison of error mean squares of each supplement (Gill, 1978). The model for LH consisted of cow and Hr considered as discrete variables.

The mathematical model for the analysis of variance of rumen VFA contents (propionate, acetate, butyrate, and total volatile fatty acid concentration as dependent variables) included only S as an independent variable.

RESULTS

Although the entire data set was analyzed through the 90 day postpartum period, only results for the 77 day postpartum period will be presented and discussed, unless specified. All cows were represented at each sampling period through day 77 postpartum, at which time the first cow was diagnosed pregnant via non-surgical embryo recovery at 18 days after mating, and further sampling was continued for all the cows that did not have an embryo recovered. Nine additional cows were diagnosed pregnant prior to 90 days postpartum. Results of statistical analyses for the entire 90 days postpartum are presented in appendix tables A-2 to A-4. The data collected at 3-day intervals are presented in appendix table A-17.

Experimental data within the 77-day period were subdivided further into two periods, defined as prior to hormone challenge (from parturition to 38 days postpartum) and posthormonal challenge (from 39 to 77 days postpartum).

Glucose Response

Postpartum decreases in glucose concentrations were described by linear polynomial regression lines (figure 1, appendix table A-11). Although overall glucose means and cumulative glucose means (CGL) did not differ due to monensin feeding for the 77-day period (table 1), cows in the monensin group had a smaller postpartum decrease than control cows ($P < .05$; table 3; figure 1). The effect of monensin

TABLE 1. LEAST SQUARES ANALYSES OF VARIANCE OF THE RESPONSE OF SUCKLED BEEF COWS DURING 77 DAYS POSTPARTUM TO A SUPPLEMENT (S) WITH OR WITHOUT MONENSIN AND TO A HORMONE (H) INJECTION (E_2 OR GnRH) AT 41 DAYS POSTPARTUM

Source of variation	P4[b]		CP4		Glucose		CGL		Error term
	df[a]	MS[a]	df	MS	df	MS	df	MS	
S	1	1608990	1	681585544	1	424.065	1	48353	Cow(SxH)
H[c]	1	3586575	1	34775159	1	0.94628	1	283341	Cow(SxH)
SxH	1	388565	1	557359039	1	1987.8	1	383533	Cow(SxH)
Cow(SxH)	24	15026696**	24	1243225571**	24	1322.38**	24	400779**	Residual
D[d]	1	105214089**	1	855827884**	1	1486.93**	1	201877761**	Residual
DxD			1	821678614**					Residual
Residual	706	2454775.39	705	46664355	693	74.66026	693	3987.7468	

**$P < .01$.

[a] df = degrees of freedom, MS = mean squares.

[b] P4 = progesterone, CP4 = cumulative P4, CGL = cumulative glucose.

[c] E_2 = estradiol-17β, GnRH = gonadotropin releasing hormone.

[d] D^2 = coded day.

TABLE 1-continued.

Source of variation	UTLO[b]		UTSIZE		UTONUS		CVX		Error term
	df[a]	MS[a]	df	MS	df	MS	df	MS	
S	1	.7643143	1	26.44149	1	0.006561	1	4.312463	Cow(SxH)
H	1	.5814455	1	156.6492	1	0.025634	1	14.98467	Cow(SxH)
SxH	1	1.036926$^+$	1	0.000682	1	0.063549	1	0.072793	Cow(SxH)
Cow(SxH)	24	.3273762**	24	229.6799**	24	0.176799**	24	51.72773**	Residual
D[c]	1	1.986699*	1	2346.795	1	0.081534	1	433.6436**	Residual
DxD	1	1.095791**	1	1280.705	1	0.093096	1	200.7335**	Residual
DxDxD	1	.7585835**	1	786.6635	1	0.174039	1	106.2494**	Residual
DxDxDxD	1	.5820033**	1	520.2613			1	61.6688**	Residual
DxDxDxDxD			1	362.2965			1	38.2192*	Residual
Residual	606	.05254352	605	43.69799	607	43.95061	605	8.694442	

$^+$P < .1, *P < .05, **P < .01.

[a] df = degrees of freedom, MS = mean squares.

[b] UTLO = uterine location, UTSIZE = uterine size, UTONUS = uterine tonus, CVX = cervix size.

[c] D = coded day.

TABLE 1-continued.

Source of variation	NFO[b]		CFO		OV		COV		NCL		CCL		Error term
	df[a]	MS[a]	df	MS	df	MS	df	MS	df	MS	df	MS	
S	1	6.3935^{+}	1	574.91^{+}	1	848.98^{+}	1	67364	1	0.1049	1	1.6378	Cow(SxH)
H	1	1.3419	1	139.62	1	2.84	1	1212	1	0.0002	1	0.0132	Cow(SxH)
SxH	1	0.8212	1	16.43	1	28.33	1	10405	1	0.2257	1	2.2507	Cow(SxH)
Cow(SxH)	24	1.9804**	24	141.11**	24	248.25**	24	29698**	24	0.1345**	24	1.5927**	Residual
D[c]	1	59.6408**	1	31.23*	1	489.11**	1	136402**	1	0.6758**	1	8.8647**	Residual
DxD			1	498.12**			1	4363**	1	1.2715**	1	15.6890**	Residual
Residual	609	0.2559	608	5.89	609	10.84	608	464	608	0.0324	608	0.28997	

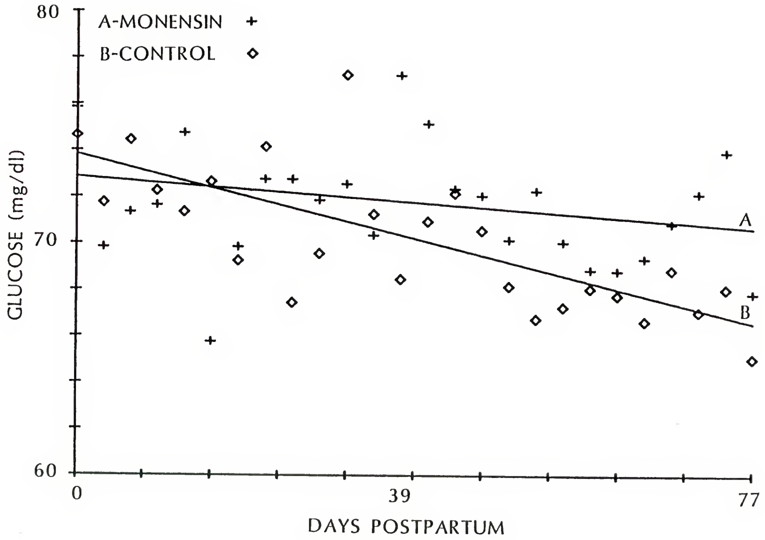
$^{+}P < .10$, $^{*}P < .05$, $^{**}P < .01$.

[a] df = degrees of freedom, MS = mean squares.

[b] NFO = no. of follicles, CFO = cumulative NFO, OV = ovarian volume, COV = cumulative OV, NCL = no. of corpora lutea, CCL = cumulative CL.

[c] D = coded day.

Figure 1. Least squares means and regressions for plasma glucose concentrations in postpartum (days 0 to 77) suckled beef cows fed a supplement with or without monensin.


80
70
60
GLUCOSE (mg/dl)
A-MONENSIN +
B-CONTROL ◇
A
B
0
39
77
DAYS POSTPARTUM

on plasma glucose concentration was not evident until approximately 41 days postpartum (figure 1). This, coincidently, was the time selected for hormonal treatments. About 4 weeks of feeding monensin (from 10 to 41 days postpartum) were needed to diminish the normal postpartum decline in plasma glucose of suckled cows. The least squares means of plasma glucose for monensin-treated and control cows were respectively 72.4 and 72.1 mg/dl ($P > .1$) during the period prior to hormonal challenge (appendix tables A-5 to A-7) and 70.2 and 68.2 mg/dl ($P > .1$) during the posthormonal challenge period (appendix tables A-8 to A-10). Monensin feeding tended to cause a higher ($P > .1$) glucose concentration during the period following hormonal challenge. Least squares means of glucose for the 77-day period are presented in table 2.

A supplement by hormone interaction was detected for CGL response curves ($P < .01$; table 3; figure 2). Monensin-treated cows injected with E_2 or GnRH had postpartum cumulations of plasma glucose that were similar. However, responses of the control cows differed more between E_2 and GnRH treatments. The supply of glucose precursors likely differed between supplemental groups because monensin increased rumen propionate concentration ($P < .01$). Propionate, besides being the major glucose precursor for ruminants (Bergman, 1973), can spare amino acids as a gluconeogenic substrate for glucose formation (Owens, 1980). Control cows injected with E_2 had a greater glucose cumulative response than control cows receiving GnRH (figure 2). This same response was evident in the analyses of the posthormonal challenge period (days 39 to 77). Therefore, in control cows E_2

TABLE 2. LEAST SQUARES MEANS OF DEPENDENT VARIABLES MEASURED DURING 77 DAYS POSTPARTUM IN SUCKLED BEEF COWS FED A SUPPLEMENT WITH (MOS) OR WITHOUT (COS) MONENSIN AND INJECTED WITH E_2 OR GnRH AT 41 DAYS POSTPARTUM

Dependent variable	MOS	COS	E_2[a]	GnRH[a]	MOSxE_2	MOSxGnRH	COSxE_2	COSxGnRH
P4 (pg/ml)	1080	1174	1057	1197	1034	1127	1081	1267
CP4 (pg/ml)	11539	13465	12284	12720	10450	12627	14119	12812
Glucose (mg/dl)	71.8	70.2	71.1	70.9	70.2	73.4	71.9	68.5
CGL (mg/dl)	958	964	981	941	955	962	1007	921
UTLO (%)	.653	.722	.717	.657	.723	.582	.712	.732
UTSIZE (mm)	60.3	60.7	60.9	59.9	60.7	59.7	61.2	60.2
UTONUS	1.09	1.09	1.1	1.08	1.11	1.07	1.09	1.1
CVX (mm)	29.3	29.1	29.3	29	29.4	29.1	29.3	28.9
NFO (unit)	0.586	0.386	0.440	0.532	0.505	0.668	0.378	0.396
CFO (unit)	4.716	2.816	3.298	4.234	4.087	5.344	2.509	3.124
OV (cm3)	14.85	12.54	13.63	13.77	14.57	15.13	12.68	12.40
COV (cm3)	166.8	146.3	155.2	157.9	161.4	172.3	148.9	143.6
NCL (unit)	0.031	0.056	0.043	0.044	0.049	0.013	0.037	0.076
CCL (unit)	0.084	0.185	0.139	0.129	0.147	0.019	0.130	0.239

[a] E_2 = estradiol-17β, GnRH = gonadotropin releasing hormone.

[b] P4 = progesterone, CP4 = cumulative P4, CGL = cumulative glucose, UTLO = uterine location, UTSIZE = uterine size, UTONUS = uterine tonus, CVX = cervix size, NFO = no. of follicles, CFO = cumulative NFO, OV = ovarian volume, COV = cumulative OV, NCL = no. of corpora lutea, CCL = cumulative NCL.

TABLE 3. TEST OF HOMOGENEITY OF REGRESSION OF THE RESPONSE OF SUCKLED BEEF COWS DURING 77 DAYS POSTPARTUM TO A SUPPLEMENT (S) WITH OR WITHOUT MONENSIN AND TO A HORMONE (H) INJECTION (E_2 OR GnRH) AT 41 DAYS POSTPARTUM

		Error term		S		Error term		H[e]		Error term		SxH	
Response	OR[a]	df[b]	MS[b]	df[c]	MS[c]	df	MS	df	MS	df	MS	df	MS
P4[d]	1	705	2457381	1	617155	705	2449213	1	6376006	703	2428410	3	8632865
CP4	2	703	46352124	2	156413569*	703	46143486	2	229749737**	699	45569251	6	17423914*
Glucose	1	692	74.1468	1	429.95*	692	74.76	1	1.331	690	74.37	3	144.5
CGL	1	692	3988	1	3470	692	3937	1	38824**	690	3726	3	64164.8**
UTLO	4	602	.0526969	4	.0194576	602	.0527447	4	.022259	594	.05231272	12	.063968
UTSIZE	5	600	44.00524	5	6.828	600	43.08746	5	116.9622*	590	43.6851	15	44.2042
UTONUS	3	604	.072762	3	.0008163	604	.0723862	3	.076452	598	.072895	9	.0040726
CVX	5	600	8.712937	5	6.47508	600	8.635179	5	15.80598	590	8.65524	15	10.2364
NFO	1	608	0.255366	1	0.57744	608	0.255862	1	0.276546	606	0.253804	6	0.678413
CFO	2	606	5.39516	2	157.38195**	606	5.636803	2	84.16475**	602	5.120552	6	83.61**
OV	1	608	10.78106	1	45.8849*	608	10.84238	1	8.6049	606	10.78522	3	21.644533
COV	2	606	408.049	2	17517.425**	606	460.4487	2	1640.385*	602	402.75314	6	6642.5567**
NCL	2	606	0.032223	2	0.0931438	606	0.032508	2	0.0064352	602	0.031372	6	0.1378785*
CCL	2	606	0.284108	2	2.06743**	606	0.290617	2	0.09552	602	0.2744595	6	1.846685**

*P < .05, **P < .01.

[a] OR = order of regression.

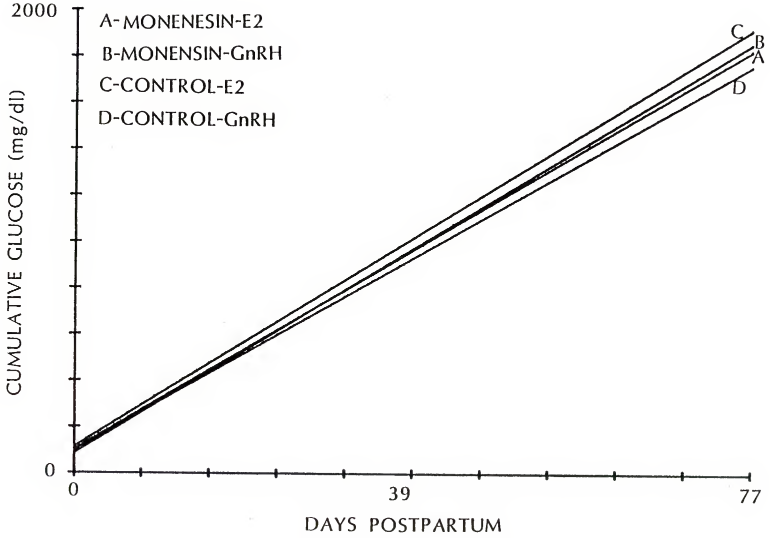
[b] Degrees of freedom (df) and mean squares (MS) associated with the error term when unpooled curves were generated for S, H, and SxH.

[c] Degrees of freedom (df) and mean square (MS) resulted from the difference of residual sums of squares of the pooled curve minus residual sums of squares of the unpooled curves.

[d] P4 = progesterone, CP4 = cumulative P4, CGL = cumulative glucose, UTLO = uterine location, UTSIZE = uterine size, UTONUS = uterine tonus, CVX = cervix size, NFO = no. of follicles, CFO = cumulative NFO, OV = ovarian volume, COV = cumulative OV, NCL = no. of corpora lutea, CCL = cumulative NCL.

[e] E_2 = estradiol-17β, GnRH = gonadotropin releasing hormone.

Figure 2. Least squares regressions of cumulative plasma glucose concentrations in postpartum (days 0 to 77) suckled beef cows fed a supplement with or without monensin and injected with either E_2 or GnRH at 41 days postpartum.


2000
0
CUMULATIVE GLUCOSE (mg/dl)
A-MONENESIN-E2
B-MONENSIN-GnRH
C-CONTROL-E2
D-CONTROL-GnRH
C
B
A
D
0
39
77
DAYS POSTPARTUM

injection may increase postpartum cumulative response of glucose. Chatterjee et al. (1979) observed that an injection of estrogen in female goats caused an increase in blood glucose concentration 24 h later under both fed and fasted conditions.

Volatile Fatty Acids

Ruminal composition of VFA was evaluated at approximately 47 days postpartum (table 4). Total VFA concentrations (monensin, 61.886 mmol/l vs Control, 58.769 mmol/l) were not affected significantly by feeding monensin. The percentage molar composition of VFA, however, was altered by monensin (table 5). Monensin reduced the acetate:propionate ratio (monensin < control; $P < .01$) due to monensin effects on molar percent of acetate (monensin < control; $P < .01$) and propionate (monensin > control; $P < .01$). Molar percent of butyrate tended to be less for monensin ($P < .06$). This change due to monensin at 47 days postpartum coincides with the higher glucose concentrations at this time (figure 1).

The observed effects of monensin on VFA concentrations are in agreement with results of Richardson et al. (1976), Moseley et al. (1977), Turner et al. (1977; 1980), McCartor et al. (1979) and Hixon et al. (1982a), who also detected an increase in propionate molar percent, a decrease in acetate molar percent and a decreased acetate:propionate ratio, with no change in total VFA concentration. However, Turner et al. (1980) observed that the total VFA concentrations were increased, compared to controls, when 200 mg of monensin were fed and decreased when 50 or 300 mg of monensin were fed. With an increase in propionate and a decrease in acetate

TABLE 4. MEAN VOLATILE FATTY ACID (VFA) CONCENTRATIONS OF RUMINAL FLUID OF SUCKLED BEEF COWS FED A SUPPLEMENT WITH OR WITHOUT MONENSIN

	VFA			
	Propionate molar %	Acetate molar %	Butyrate molar %	Total Concentration mmoles/l
Monensin	25.6	64.6	10.5	61.886
Control	20	68.6	11.4	58.769

TABLE 5. LEAST SQUARES ANALYSES OF VARIANCE OF VOLATILE FATTY ACID OF SUCKLED BEEF COWS FED A SUPPLEMENT (S) WITH OR WITHOUT MONENSIN FOR 37 DAYS

	Acetate		Propionate		AC:PRO[a]		Butyrate		Total concentration	
Variable	df[b]	MS[b]	df	MS	df	MS	df	MS	df	MS
S	1	0.013464**	1	0.020961**	1	5.95208**	1	0.000572+	1	65.4692
Residual	25	0.000249	25	0.000367	25	0.098109	25	0.000153	25	167.1009

+ $P < .10$; ** $P < .01$.

[a] acetate:propionate ratio.

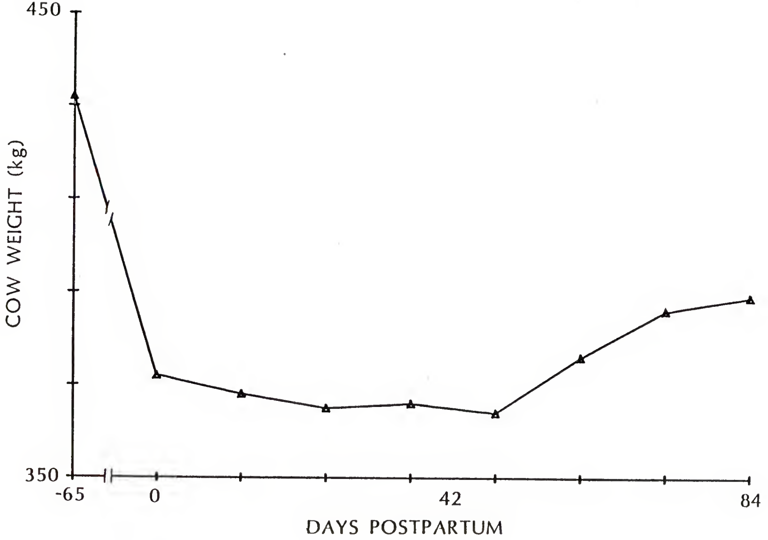
[b] df = degrees of freedom; MS = mean squares.

production, there is a reduction in loss of energy as methane and an improved fermentation efficiency (Owens, 1980).

An evaluation of body weights throughout the postpartum period failed to detect an effect of feeding monensin during the postpartum period in suckled cows. The average condition score by 41 days postpartum was 4.8, utilizing a scale of 1 to 17, and no differences were detected between supplements. A condition score of 4 was assigned to healthy cows that had no fat deposits observed visually and a score of 8 was given to cows that had an estimated .20" to .25" of fat over the 12-13th ribs (Andrade, 1980).

A loss of 60 kg in body weight occurred from prepartum (432 kg; ± 65 days before calving; condition score 5.9) to 1 day postpartum (372 kg; figure 3). The average calf birth weight was 36 kg and represented 60% of the loss of maternal weight from before parturition to 1 day postpartum. The pre- to 24 h postpartum loss of 60 kg in body weight was close to that observed by Rawlings et al. (1980; 48 kg) and Humphrey et al. (1983; 55 kg). Cows lost about 2% of their body weight from parturition (372 kg) to about 50 days postpartum (366 kg); at 84 days postpartum body weight increased to 390 kg, which was higher than their first postpartum weight (372 kg; figure 3). This indicates that cows were not at their heaviest weight at the time of parturition. This probably reflects a negative energy balance during the prepartum period even though cows were supplemented as a group with about 3 kg of ground corn and 6.8 kg of hay per head daily, on pasture, before calving. Somerville et al. (1979) observed that cows fed different levels of their estimated energy requirement for maintenance (175, 125 and 90%) lost 8, 16 and 21% of their calving

Figure 3. Overall actual means of prepartum (day 65) and postpartum (days 0 to 84) body weight of suckled beef cows.


450
350
COW WEIGHT (kg)
-65
0
42
84
DAYS POSTPARTUM

weight by 100 day postpartum. They had conception rates of 94, 91 and 82%, respectively. Andrade (1980) reported that weight changes of two-year-old heifers during the last 60 days of gestation were 7.8, -6.2 and -24 kg with subsequent pregnancy rates of 80 ± 8, 82 ± 8 and 71 ± 8%, respectively.

There was no difference in average daily weight gain of calves due to feeding monensin to their dams (.62 vs .68 kg/day). Calves of cows fed monensin weighed 90.7 kg while calves of control cows weighed 98.8 kg at the end of experiment. This also has been reported by others (Shalles and Fleck, 1979; Turner et al., 1980; and Walker et al., 1980a).

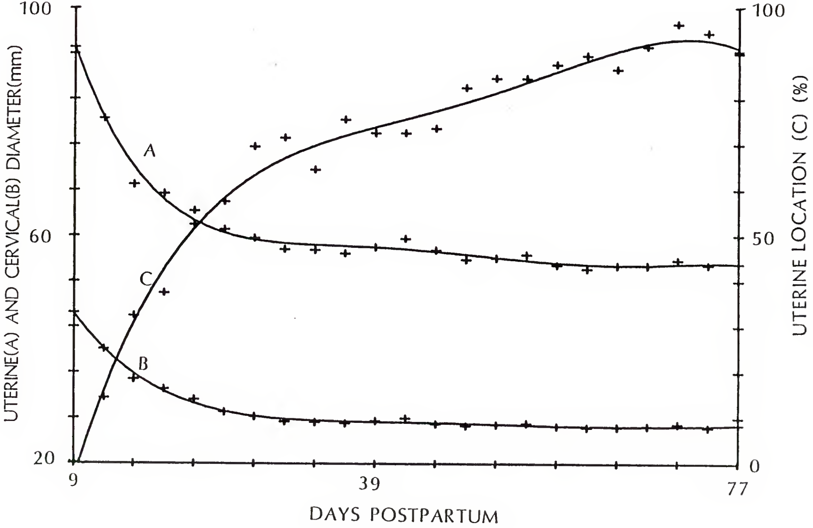
Although monensin altered glucose concentration and VFA molar percentage, these metabolic differences were not associated with differences in maternal body weight or calf growth.

Uterine Involution

Neither the feeding of monensin nor hormone (GnRH or E_2) had a major effect on uterine or cervical measurements in suckled beef cows (table 1). This agrees with earlier observations that energy level had no detectable effect on uterine involution in dairy cows (Oxenreider and Wagner, 1971) or in suckled beef cows (Wiltbank et al., 1962; Dunn et al., 1969).

The rapid decrease in uterine diameter from day 9 to day 15 (figure 4) is in agreement with Morrow et al. (1966), Gier and Marion (1968), and Guibault (personal communication). Cervical and uterine diameters decreased in a parallel manner (figure 4), were described by a fifth order regression (appendix table A-12), and were highly correlated ($r = .90$, $P < .01$). This association indicates that

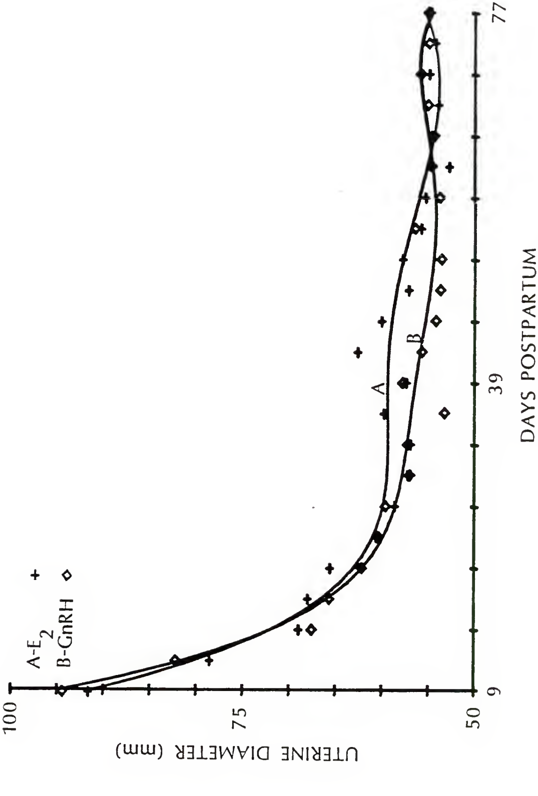
Figure 4. Least squares means and regressions for uterine (A) and cervical diameter (B) and uterine location (C) in postpartum (days 9 to 77) suckled beef cows.


100
60
20
UTERINE(A) AND CERVICAL(B) DIAMETER(mm)
A
C
B
9
39
77
DAYS POSTPARTUM
100
50
0
UTERINE LOCATION (C) (%)

involution of the cervix is a good indicator of uterine involution. Fonseca et al. (1983) reported that involutions of the uterus and cervix were closely related in Holstein and Jersey cows. Guibault (personal communication) also observed that uterine and cervical involution were correlated significantly in Brown Swiss cows. The measurement of cervix diameter between days 12 and 26 postpartum was a good indicator of subsequent reproductive performance of milked cows (Oltenacu et al., 1983). Gross involutions of the uterus and cervix were completed by approximately 30 days postpartum, since further changes in size were minimal.

The change in location of the uterus from abdominal cavity to pelvic cavity was most evident from 9 to 30 days postpartum when about 70% of the uteri were already in the pelvic cavity (figure 4). Uterine and cervical involutions were negatively correlated with uterine location ($r = -.66$ and $-.66$; $P < .001$), respectively. Uterine tonus increased gradually up to 77 days postpartum. A transitory increase in tonus was observed from 40 to 54 days postpartum and was associated with a transitory increase in uterine diameter after hormonal challenge with E_2. Day trends for uterine size differed ($P < .05$) between hormonal treatments for the entire 77-day postpartum period (table 3, figure 5) and for the posthormonal challenge period ($P < .001$; appendix table A-10). Injection of E_2 probably caused an increase in uterine size due to increased blood flow, edema and cell proliferation (Asdell et al., 1949). Marion et al. (1968) indicated that estrogen injection at a physiological dose may delay uterine involution by increasing size of the uterine wall. Since E_2 is a long-acting steroid hormone, it can induce true

Figure 5. Least squares means and regressions of uterine diameter in postpartum (days 9 to 77) suckled beef cows injected with either E_2 or GnRH at 41 days postpartum.


A-E_2 +
B-GnRH ◇
A
B
UTERINE DIAMETER (mm)
100
75
50
9
39
77
DAYS POSTPARTUM

uterine growth via long-term retention of the estrogen-nuclear receptor complex in the nucleus. This retention is responsible for induction of a second rise in polymerase II activity as an obligatory step in production of true uterine growth (Clark et al., 1977). It was interesting that these potential biomolecular effects resulted in a uterine response detected by palpation. Uterine size tended to decrease for a few days following injection of GnRH (figure 5). Britt et al. (1974) reported that cows injected with GnRH much earlier in the postpartum period (at 14 days postpartum) had an enhanced rate of uterine involution. This was later confirmed by Fernandes et al. (1978b). This small decrease in uterine size due to GnRH injection may be due to an increase in P_4 concentrations as suggested by Britt et al. (1974).

Ovarian Activity

Gross ovarian changes, evaluated by rectal palpation during the first 38 days postpartum, indicated that monensin-treated cows had a greater mean number of follicles (NFO; $P < .05$) and a greater mean cumulative number of follicles (CFO; $P < .05$) than control cows (appendix table A-5). It is possible that the monensin effect on NFO observed during the first 38 days postpartum ($P < .05$) persisted for the entire 77-day postpartum period (figure 6),since monensin had no significant effect on mean NFO during the posthormonal challenge period (days 39 to 77; appendix table A-8), and the monensin effect on mean NFO approached significance ($P < .1$) during the entire 77 days postpartum (table 1).

It is clear that postpartum day trends for number of follicles are similar throughout the entire postpartum period (table 3), but monensin-treated cows had a higher number of follicles ($P < .05$;

Figure 6. Least squares means and regressions of number of follicles in postpartum (days 9 to 77) suckled beef cows fed a supplement with or without monensin.

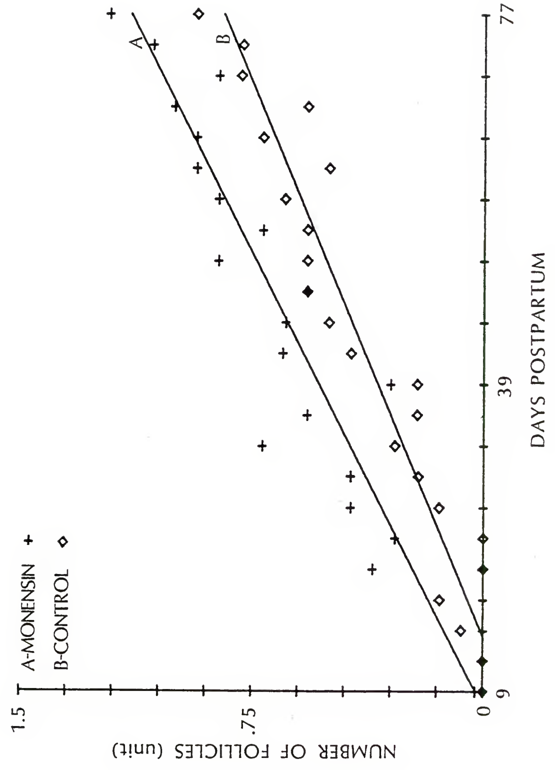

A-MONENSIN +
B-CONTROL ◇
NUMBER OF FOLLICLES (unit)
1.5
.75
0
9
39
77
DAYS POSTPARTUM
A
B

table 1; figure 6). Gonadotropin releasing hormone, which releases both LH (Reeves et al., 1971; Akbar et al., 1974; Britt et al., 1974; Kaltenbach et al., 1974; Schams et al., 1974; Echternkamp, 1978; Fernandes et al., 1978a; Foster et al., 1980; figure 16) and FSH (Akbar et al., 1974; Kaltenbach et al., 1974; Schams et al., 1974; Foster et al., 1980), has the potential to stimulate follicle development. Since follicle development was greater in the monensin group (days 9 to 38; $P < .05$; appendix table A-5), it was interesting that GnRH given to monensin-treated cows caused a greater stimulatory response in number of follicles developed through the 77-day postpartum period than in monensin-treated cows injected with E_2 (figure 7). Furthermore, the stimulatory effect of GnRH was not as great in control cows. Test for homogeneity of regression (table 3) indicated that there was a supplement by hormone interaction ($P < .01$) for the postpartum response curves for cumulative number of follicles (CFO; figure 7). The GnRH challenge induced a greater follicular response in the monensin group compared to the control group. This interaction (SxH) was also significant for the posthormonal challenge period (days 39 to 77; appendix table A-10).

Ovarian changes in number of follicles is likely to influence measurements of ovarian volume. This was indeed the case, since the postpartum day trends for ovarian volume were not parallel between the monensin and control groups ($P < .05$; table 3; figure 8) and the number of follicles was correlated with ovarian volume ($r = .33$; $P < .01$). The monensin group had a greater postpartum (days 9 to 77) increase in ovarian volume (figure 8). This difference due to monensin also was reflected by the different day trends for

Figure 7. Least squares means and regressions of cumulative number of follicles in postpartum (days 9 to 77) suckled beef cows fed a supplement with or without monensin and injected with either E_2 or GnRH at 41 days postpartum.

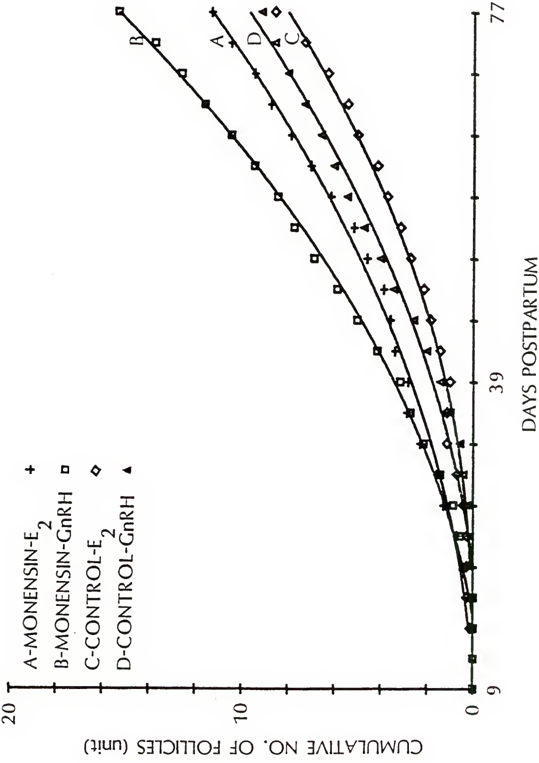
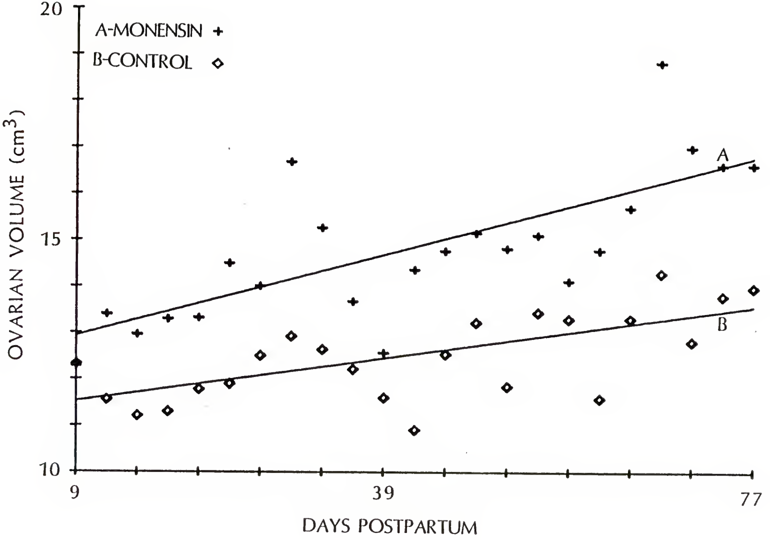

A-MONENSIN-E_2 +
B-MONENSIN-GnRH □
C-CONTROL-E_2 ◇
D-CONTROL-GnRH ▲
CUMULATIVE NO. OF FOLLICLES (unit)
20
10
0
9
39
77
DAYS POSTPARTUM
B
A
D
C

Figure 8. Least squares means and regressions of ovarian volume in postpartum (days 9 to 77) suckled beef cows fed a supplement with or without monensin.


A-MONENSIN +
B-CONTROL ◇
OVARIAN VOLUME (cm³)
20
15
10
9
39
77
DAYS POSTPARTUM
A
B

cummulative response of ovarian volume ($P < .01$; table 3; figure 9). In part, the interactions between supplement and hormone for postpartum day trends of ovarian volume cumulative response ($P < .01$; table 3; figure 10) agreed with the follicular responses, in that GnRH elicited a greater response as compared to E_2, on ovarian volume in monensin-treated cows compared to control cows.

Monensin-treated cows generally had larger ovaries, which was probably in part due to a greater number of follicles during the 77-day postpartum period. This was supported by a correlation of .33 ($P < .01$) between number of follicles and ovarian volume. Ovarian response to hormonal challenge was affected by monensin feeding, since monensin-treated cows injected with GnRH had a greater follicular response than those injected with E_2. It is interesting to note that monensin-treated cows had larger ovarian volumes very early after monensin feeding began (3 to 6 days; figure 8). This difference continued to increase up to 77 days postpartum. The same general trend was observed for number of follicles (figure 6). These observations indicate that monensin may have a direct effect on the ovary since ovarian volume seems to change very rapidly after the start of monensin treatment, and a shift in the rumen VFA content that could cause these differences probably would take longer. Monensin can increase the intercellular concentration of Na, thus there is a possibility that an increased concentration of intercellular Na could cause the ovarian cells to absorb more water and increase in size. Austic and Smith (1980) pointed out that differences in the cellular response to the ionophores may relate to their influence on Na-K pump activity and their relative affinities

Figure 9. Least squares means and regressions of cumulative ovarian volume in postpartum (days 9 to 77) suckled beef cows fed a supplement with or without monensin.

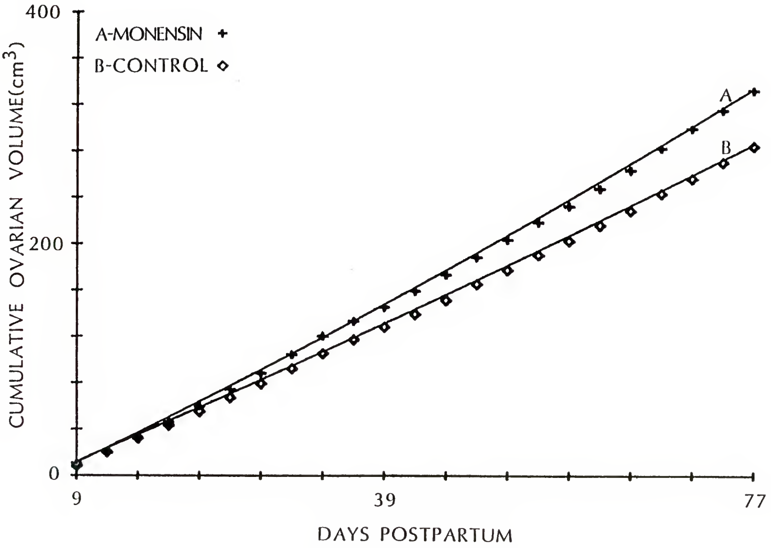
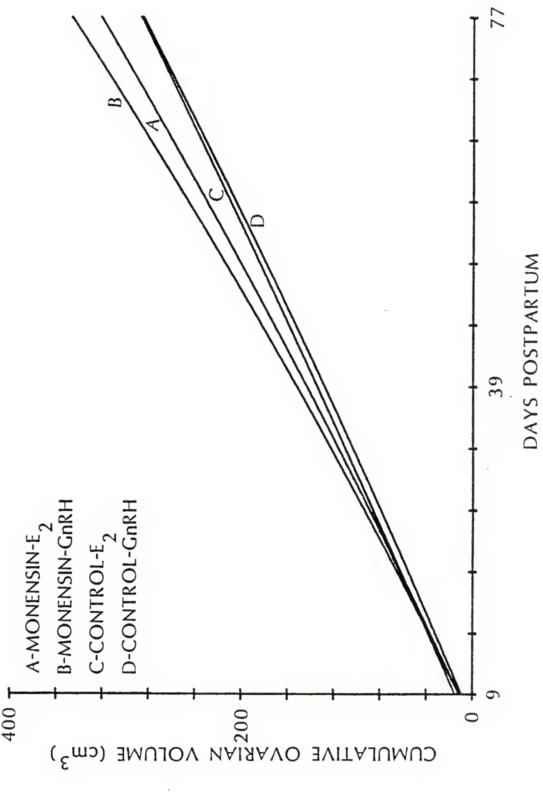

A-MONENSIN +
B-CONTROL ◇
CUMULATIVE OVARIAN VOLUME(cm3)
400
200
0
9
39
77
DAYS POSTPARTUM
A
B

Figure 10. Least squares regressions of cumulative ovarian volume in postpartum (days 9 to 77) suckled beef cows fed a supplement with or without monensin and injected with either E_2 or GnRH at 41 days postpartum.


A-MONENSIN-E_2
B-MONENSIN-GnRH
C-CONTROL-E_2
D-CONTROL-GnRH
B
A
C
D
CUMULATIVE OVARIAN VOLUME (cm^3)
400
200
0
9
39
77
DAYS POSTPARTUM

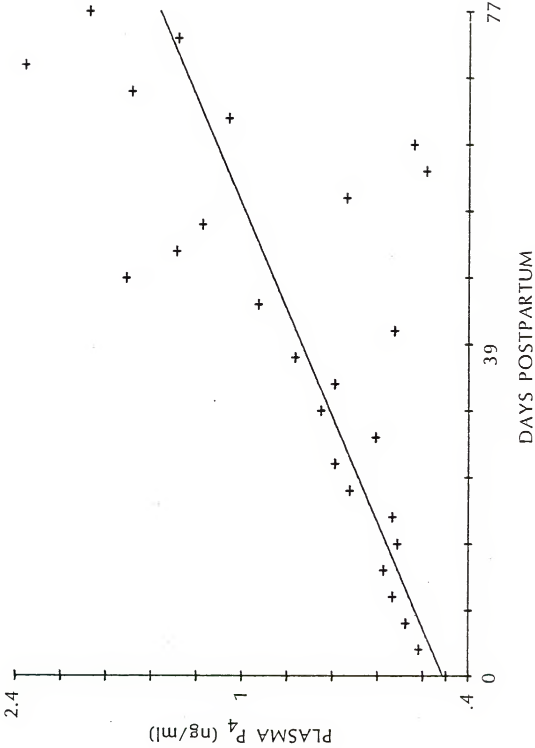
for various inorganic and organic ions. It is yet to be determined if ovarian cells are sensitive to monensin. Bushmich et al. (1980) reported that prepuberal heifers fed monensin had larger ($P < .025$) ovaries 15 days after feeding began but no measurements were made before day 15.

Progesterone

Plasma concentrations of P_4 have been utilized as a measurement of ovarian activity during the postpartum period (Edgerton and Hafs, 1973; Webb et al., 1977; Fernandes et al., 1978a; Berardinelli et al., 1979; Kesler et al., 1980; Lamming et al., 1981; Manns et al., 1983). Progesterone concentrations greater than 1 ng/ml are an indication that luteal like tissue is present within the ovary (Castenson, 1974; Manns et al., 1983). Concentrations of P_4 increased throughout the postpartum period in suckled cows (figure 11). However, mean concentrations of P_4 did not exceed 1 ng/ml until approximately 39 days or just prior to the time of hormonal challenge. After 40 days postpartum, a distinct cyclic pattern in P_4 concentrations was observed and appeared to be attributed to the hormonal treatments (GnRH and E_2; figure 11).

Palpable CL were detected in only seven of 28 cows within the 77-day postpartum period. It has been demonstrated that first formed or induced CL in the early postpartum period are often embedded within the ovary and not detectable via rectal palpation (Castenson, 1974; Pratt et al., 1982). Berardinelli et al. (1979) also observed that the source of a short-term (7 ± 2 days) increase in P_4 concentrations before puberty was a compact luteal tissue embedded in the ovary but not palpable or observable grossly on the ovarian surface.

Figure 11. Least squares means and regression of plasma progesterone concentration in postpartum (days 9 to 77) suckled beef cows.


PLASMA P_4 (ng/ml)
2.4
1
.4
0
39
77
DAYS POSTPARTUM

Consequently, it was difficult to accurately detect newly formed CL following hormonal treatment at 41 days postpartum. Progesterone concentrations of the cows that started cycling within 77 days postpartum are shown on appendix figure A-3.

Four cows began cycling prior to hormonal challenge, based on a detected estrus followed by an increase in P_4 concentrations at the time of hormonal treatment (GnRH or E_2). Of the remaining 12 noncycling cows in each of the GnRH and E_2 groups, 50% (6 cows per group) had an increase in P_4 within 6 days following hormonal challenge. For example, five cows within the GnRH group had P_4 concentrations exceeding 1 ng/ml for up to 6 days, and one cow had P_4 concentrations as high as 9 ng/ml with values greater than 1 ng/ml for a 13-day period. Although an induced ovulation was likely no CL was detected by palpation. Six noncycling cows of the E_2 group had concentrations of P_4 between 1 and 3 ng/ml for a period of 3 to 6 days. All noncycling cows of the E_2 group came into heat within 50 h after E_2 injection. A CL was not detected via rectal palpation in any of the 12 noncycling cows expressing a plasma P_4 increase after hormonal challenge.

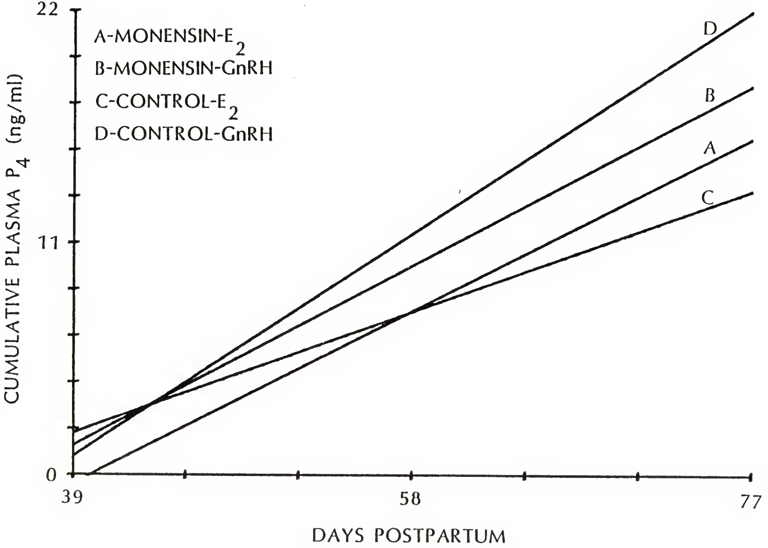
Various authors have reported an increase in P_4 and(or) ovulation after GnRH injection in cows (Castenson, 1974; Britt et al., 1974; 1975; Kesler et al., 1977; 1980; Webb et al., 1977; Lishman et al., 1979; Troxel et al., 1980; Wetteman et al., 1982). Luteinization of either existing follicles or cystic follicles has been suggested as contributing to the increase in P_4 concentrations after GnRH challenge (Castenson, 1974; Corah et al., 1974; Kesler et al., 1980; Webb et al., 1980). Thus, it is possible that the LH increase caused by GnRH or E_2 injection (figures 16-17) was responsible for ovulation or luteinization of existing follicles. This would account for the transitory increase in concentrations of P_4. Fonseca et al. (1980) reported that two of 14 suckled cows had normal estrous cycle after the first injection of GnRH.

After the transitory increase in P_4 following the hormonal challenge, more cows started cycling and mean P_4 concentrations tended to be higher than 1 ng/ml (figure 11).

During the period following hormonal challenge, cows treated with GnRH secreted more P_4 than cows challenged with E_2. This occurred regardless of whether cows were fed a supplement with or without monensin (figure 12). Although monensin-treated cows challenged with GnRH had greater follicular response, the control cows challenged with GnRH secreted more P_4. This indicates that increased follicular activity due to feeding monensin does not necessarily insure a greater frequency of ovulation or luteal activity after a GnRH injection.

Test of homogeneity of regression indicated that there was a supplement by hormone interaction for the period following hormonal challenge ($P < .01$; figure 12, appendix table A-10). The GnRH injection stimulated a greater secretion of P_4 in control cows than in monensin-treated cows (figure 12). Estradiol 17-β injection also stimulated P_4 production. It was observed that one control cow, that was in heat 5 days before E_2 injection, had a short estrous cycle (16-day interval) and stopped cycling after the second postpartum estrus. This could be the cause of the small rate of P_4 cumulation observed for control cows injected with E_2 (figure 12); however, when this cow and the other three cows that started cycling

Figure 12. Least squares regressions of cumulative plasma progesterone concentrations in postpartum (days 39 to 77) suckled beef cows fed a supplement with or without monensin and injected with either E_2 or GnRH at 41 days postpartum.


22
11
0
CUMULATIVE PLASMA P_4 (ng/ml)
A-MONENSIN-E_2
B-MONENSIN-GnRH
C-CONTROL-E_2
D-CONTROL-GnRH
D
B
A
C
39
58
77
DAYS POSTPARTUM

before hormonal challenged were dropped from the analyses, control cows injected with E_2 still secreted less P_4 than control cows injected with GnRH and monensin-treated cows injected with GnRH or E_2 during the posthormonal challenge period (figure 13). Thus, a GnRH challenge stimulated a greater secretion of P_4 than did an E_2 challenge. It is possible that an earlier and better synchronized release of LH due to GnRH stimulates greater P_4 secretion than LH response due to E_2. Estrogen injection has been shown to caused regression of the CL in cycling, pregnant and hysterectomized heifers (Wiltbank, 1966). Bolt et al. (1971) observed that estrogen had a luteolytic effect in intact ewes injected on day 10 of the cycle but not in hysterectomized ewes or when administered on day 3 of the estrous cycle. It has been shown that estrogen injection induced an LH surge (Swanson and McCarthy, 1978; Short et al., 1979; Forrest et al., 1980b; Echternkamp et al., 1982). Thus, the GnRH induced synchronized surge of the luteotropic hormone LH may influence P_4 production and ovarian response in a better way than the less synchronized LH surge induced by estrogen. Another difference is that estrogen has a luteolytic effect as shown by Wiltbank (1966) in cattle and Bolt et al. (1971) in ewes.

Luteinizing Hormone

There was a tendency for plasma concentrations of LH to increase ($P > .1$) in a linear fashion from day of parturition (.06 ng/ml) to day 38 postpartum (.18 ng/ml; figure 14). This early postpartum increase in plasma LH agrees with other reports (Edgerton and Hafs, 1973; Kesler et al., 1977; 1980; Fernandes et al., 1978a; Goodale et al., 1978; Jordan and Swanson, 1979; Stevenson and Britt, 1979; Humphrey et al., 1983). A concurrent increase in basal P_4

Figure 13. Least squares regression of cumulative plasma progesterone concentrations in postpartum (days 39 to 77) suckled beef cows fed a supplement with or without monensin that were in anestrous before injection of either E_2 or GnRH at 41 days postpartum.

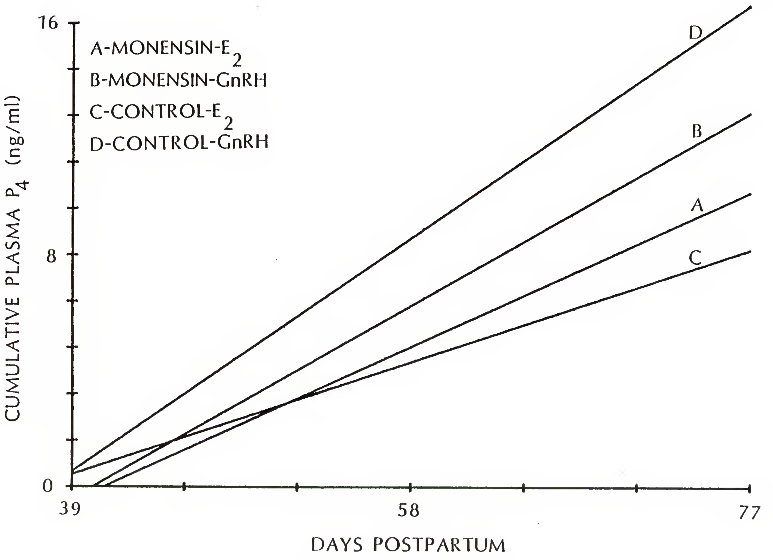

16
8
0
39
58
77
CUMULATIVE PLASMA P_4 (ng/ml)
DAYS POSTPARTUM
A-MONENSIN-E_2
B-MONENSIN-GnRH
C-CONTROL-E_2
D-CONTROL-GnRH
D
B
A
C

Figure 14. Least squares means and regression of plasma luteinizing hormone concentrations in postpartum (days 0 to 38) suckled beef cows.

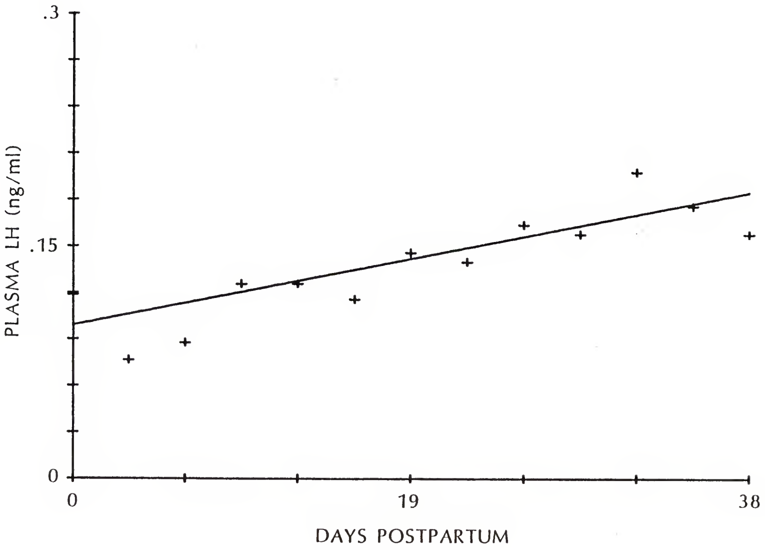
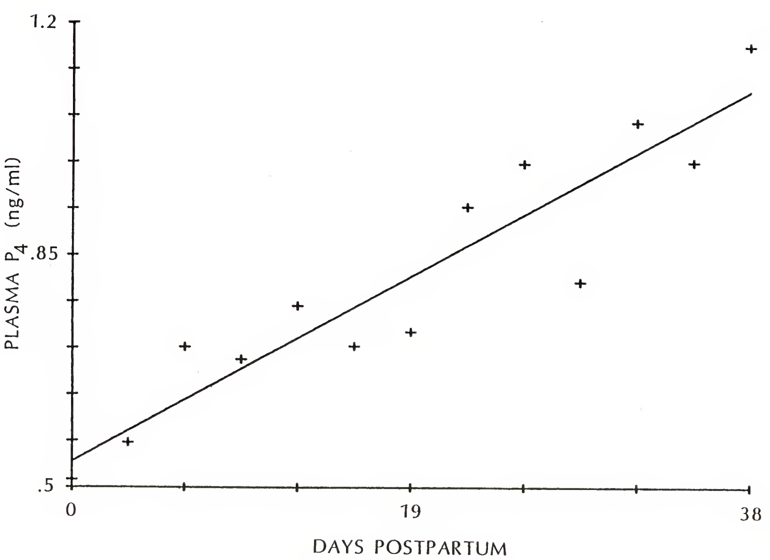

PLASMA LH (ng/ml)
.3
.15
0
0
19
38
DAYS POSTPARTUM

Figure 15. Least squares means and regression of plasma progesterone concentrations in postpartum (days 0 to 38) suckled beef cows.


1.2
.85
.5
PLASMA P_4 (ng/ml)
0
19
38
DAYS POSTPARTUM

concentration (figure 15) also was detected from the day of parturition (± .5 ng/ml) to 38 days postpartum (± 1 ng/ml). Increasing basal concentrations of plasma LH may have caused some luteinization of follicular granulosa cells (Sherwood and McShan, 1977). The mean plasma P_4 concentrations were not sufficient ($<$ 1 ng/ml) to indicate the presence of luteal tissue. The increase in plasma LH concentrations may be due to an increase in pituitary synthesis of LH, as represented by a postpartum increase in pituitary LH content (Saiduddin et al., 1968; Wagner et al., 1969; Echternkamp, 1978). Secretion of LH does increase during this period, as represented by an elevation in frequency and magnitude of LH peaks (Rawlings et al., 1980; Humphrey et al., 1983). In the present experiment, the sample interval was not frequent enough to characterize these changes. Stevenson and Britt (1979) suggested that follicular estrogen secretion exerted a positive neuroendocrine feedback to stimulate LH secretion.

Monensin feeding did not alter the mean basal concentrations of LH when measured every 3 days up to 38 days postpartum (one sample per day; appendix tables A-5 to A-7). Mean basal LH concentrations did not differ due to monensin (tables 6 and 7), when measured for 4 h at 20 min intervals before GnRH or E_2 injection at about 41 days postpartum. Randel and Rhodes (1980) also did not detect differences in mean LH concentrations before GnRH challenge between prepuberal heifers fed monensin and those fed a control ration. In the present study, however, basal LH values were more variable in monensin than in control cows (table 8). Cows were responsible for 93.7% of the total LH variation in the monensin group and 86.5% of the total variation in control group, with intraclass correlations of .97 and .92,

TABLE 6. LEAST SQUARES ANALYSIS OF VARIANCE OF THE PRECHALLENGE BASAL LH CONCENTRATIONS OF SUCKLED BEEF COWS FED A SUPPLEMENT (S) WITH OR WITHOUT MONENSIN

Source of variation	LH[a] df[b]	LH[a] MS[b]	Error term
S	1	.0075703	cow (S)
Cow (S)	26	4.3750714**	residual
Hr[c]	1	.0009497	residual
Residual	335	6.6843033	

** P < .01.
[a] LH = luteinizing hormone.
[b] df = degrees of freedom; MS = mean squares.
[c] Hr = hour (samples taken very 20 min for 4 h).

TABLE 7. TEST OF HOMOGENEITY OF REGRESSION OF THE PRECHALLENGE BASAL LH CONCENTRATION OF SUCKLED BEEF COWS FED A SUPPLEMENT (S) WITH OR WITHOUT MONENSIN

		Error Term		S	
Response	OR[a]	df[b]	MS[b]	df[c]	MS[c]
LH	1	334	.00685316	1	.0117558

[a] OR = order of regression.

[b] Degrees of freedom (df) and mean squares (MS) associated with the error term when unpooled curves for time trend were generated for S.

[c] Degrees of freedom (df) and mean squares (MS) obtained from the difference of residual sums of squares of the pooled time curve minus residual sums of squares of the unpooled time curves.

TABLE 8. LEAST SQUARES ANALYSES OF VARIANCE OF THE BASAL LH CONCENTRATIONS BEFORE HORMONAL CHALLENGE IN SUCKLED BEEF COWS FED A SUPPLEMENT WITH (MOS) OR WITHOUT (COS) MONENSIN

Source of variation	MOS		COS		Error term
	df[b]	MS[b]	df	MS	
Cow	13	0.2642**	13	0.0723**	Residual
Hr[c]	12	0.0103	12	0.0059	Residual
Residual[d]	156	0.00778	156	0.00572	

**P < .01.

[a] Samples were collected every 20 min.

[b] df = degrees of freedom, MS = mean squares.

[c] Hr = hour, (samples taken every 20 min for 4 h).

[d] the residual mean squares (RMS) were used to test the variance between the basal LH concentrations of cows fed a supplement with (RMS_m) or without (COS) monensin (RMSc) RMSm/RMSc=f where $f\alpha/2, r_1-1, r_2-1$ where r = no. of observations per treatment. f = 0.00778/0.00572 = 1.36, where $f_{155,155} \approx 1.27$.

respectively. The greater variability in basal LH for the monensin group probably reflects the greater ovarian activity of the monensin-treated cows by the time of hormonal challenge. A monensin induced increase in follicle growth and perhaps estradiol production may exert a positive feedback to increase the basal secretion of LH or enhance concentration of plasma LH (Stevenson and Britt, 1979).

Luteinizing Hormone Response to Injection of GnRH or E_2

Response to GnRH

The LH response to injections of GnRH was described by a fifth order polynomial regression (appendix table A-13). Mean concentrations of LH did not differ for monensin vs control group (3.0 vs 4.4) following injection of GnRH (tables 9 and 10). However, the LH response curves to GnRH differed ($P < .01$) between groups (table 11). Cows in the control group released more LH after GnRH injection than those fed monensin (table 11; figure 16). Luteinizing hormone concentration started to increase within 5 minutes after GnRH challenge and reached a maximum mean volume of 11.4 ng/ml by 120 min in control cows and 7.5 ng/ml by 150 min in monensin-treated cows. Two cows, one in each supplemental group, came into estrus at days 9 and 3 before GnRH challenge. Their maximum values of LH were 4.9 ng/ml (monensin) and 6.7 ng/ml (control). These values were within the range of maximum LH concentrations observed in anestrous cows fed the supplement with (3.2 to 15.4 ng/ml) or without monensin (5.1 to 26.7 ng/ml).

Mean basal concentrations of LH prior to injection of GnRH were correlated with mean LH concentrations after challenge ($r = .56$; $P < .05$; no. = 14) and with a peak LH value ($r = .52$; $P < .06$; no. = 14).

TABLE 9. LEAST SQUARES MEANS OF LH CONCENTRATIONS (ng/ml) MEASURED BEFORE AND AFTER E_2 OR GnRH INJECTION IN SUCKLED BEEF COWS FED A SUPPLEMENT WITH (MOS) OR WITHOUT (COS) MONENSIN

Variable	MOS	COS
LH[a] (before)	0.24	0.23
LH (after GnRH[b])	3.04	4.46
LH (after E_2[b])	0.68	0.5464

[a] LH = luteinizing hormone.
[b] E_2 = estradiol-17β, GnRH = gonadotropin releasing hormone.

TABLE 10. LEAST SQUARES ANALYSES OF VARIANCE OF THE LH RESPONSE OF SUCKLED BEEF COWS TO A SUPPLEMENT (S) WITH OR WITHOUT MONENSIN AND INJECTED WITH E_2 OR GnRH AT 41 DAYS POSTPARTUM

Source of variation	GnRH[a]		E_2[b]		Error term
	df[c]	MS[c]	df	MS	
S	1	153.2	1	1.87	Cow(S)
Cow(S)	12	1527.25**	12	14.69**	Residual
Hr[d]	1	23.94**	1	2.85	Residual
HrxHr	1	168.08**	1	6.09	Residual
HrxHrxHr	1	202.19**	1	10.03+	Residual
HrxHrxHrxHr	1	179.54**	1	14.88*	Residual
HrxHrxHrxHrxHr		147.44**	1	21.07**	Residual
Residual	288	6.077	415	2.80	

+P < .10, *P < .05, **P < .01.

[a] GnRH = gonadotropin releasing hormone.

[b] E_2 = estradiol-17β.

[c] df = degrees of freedom, MS = mean squares.

[d] Hr = hour after challenge.

TABLE 11. TEST OF HOMOGENEITY OF REGRESSION OF THE LH RESPONSE OF SUCKLED BEEF COWS TO A SUPPLEMENT (S) WITH OR WITHOUT MONENSIN AND INJECTED WITH E_2 OR GnRH AT 41 DAYS POSTPARTUM

		GnRH[a]				E_2[b]				
		Error term		S			Error term		S	
Response	OR[c]	df[d]	MS[d]	df[e]	MS[e]	OR	df	MS	df	MS
LH[f]	5	283	5.6909	5	27.9326**	5	410	2.8032	5	2.2657

**P < .01.

[a] GnRH = gonadotropin releasing hormone.

[b] E_2 = estradiol-17β.

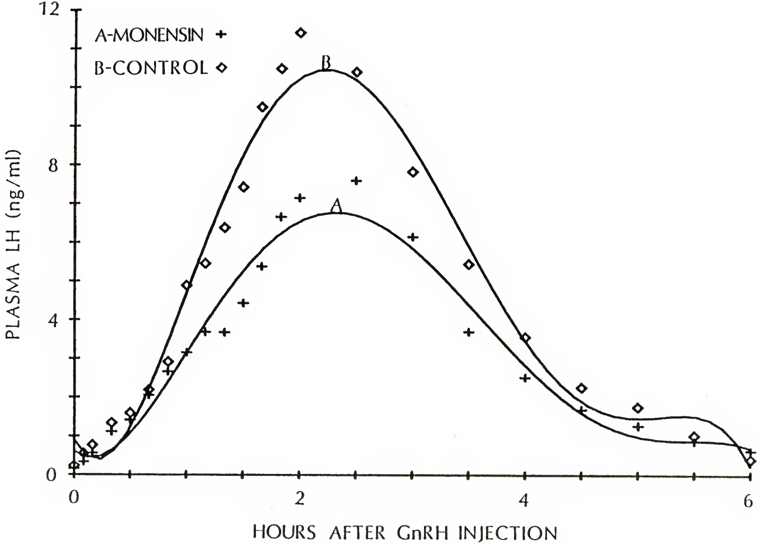
[c] OR = order of regression.

[d] Degrees of freedom (df) and mean square (MS) associated with error term when unpooled curves were generated for S.

[e] Degrees of freedom (df) and mean square (MS) resulted from the difference of residual sums of square of the pooled curve minus residual sums of square of the unpooled curves.

[f] LH = luteinizing hormone.

Figure 16. Least squares means and regressions of plasma luteinizing hormone responses to injections (on day 41 postpartum) of GnRH (100 ug) in postpartum suckled beef cows fed a supplement with or without monensin.


A-MONENSIN +
B-CONTROL ◇
B
A
PLASMA LH (ng/ml)
12
8
4
0
0
2
4
6
HOURS AFTER GnRH INJECTION

Response to E_2

Feeding of monensin had no effect on LH means (table 10), and the LH response curves of cows fed monensin did not differ from those of cows fed a supplement without monensin (table 11). The LH response due to E_2 was described by a fifth order polynomial regression (figure 17; appendix table A-13). Plasma LH concentrations started to increase by approximately 24 h after E_2 injection. However, bleeding was terminated at 30 h after injection of E_2, and characterization of the entire LH response was not possible. Seven of the 14 cows injected with E_2 (3 in the monensin and 4 in the control group) had the beginning of an induced LH rise within 30 h post E_2 injection. At 30 h, LH concentrations for the seven cows that had a rise in LH were 25.6, 2.1 and .33 ng/ml for the monensin group and 15.6, 7.9, 7.9 and 8.4 ng/ml for the control group. One monensin cow injected with E_2 had a complete LH surge. This was one of two cycling cows and she was injected on day 4 of the estrous cycle. A maximum LH value of 2.4 ng/ml was detected at 25 h after E_2 injection, and at 30 h the values had returned to basal concentrations (.33 ng/ml; figure 18). The LH concentrations at 30 h for the cows that did not have an increase in LH were .45, .5, .43 and .75 (cycling cow) ng/ml for the monensin group and .7, .35 and .55 ng/ml for control group. Bolt et al. (1971) reported that ewes injected with estrogen on the third day of the cycle were able to have an induced LH surge. Probably the concentrations of P_4 and(or) time under P_4 influence were not sufficient to inhibit an LH surge induced by E_2 in the cycling cow. An induced LH response did not occur in the middle of the estrous cycle in cows or heifers (Hobson and Hansel, 1972; Short et al.,

Figure 17. Least squares means and regression of plasma luteinizing hormone response to injections (on day 41 postpartum) of estradiol-17β (10 mg) in postpartum suckled beef cows.

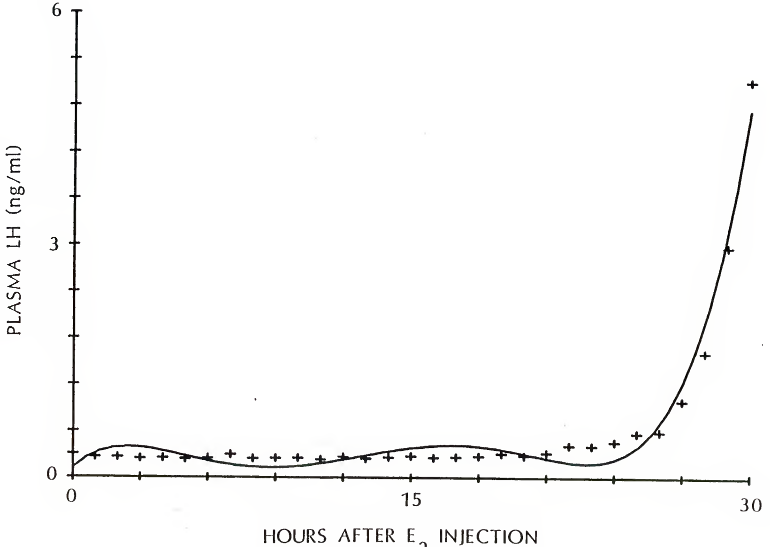
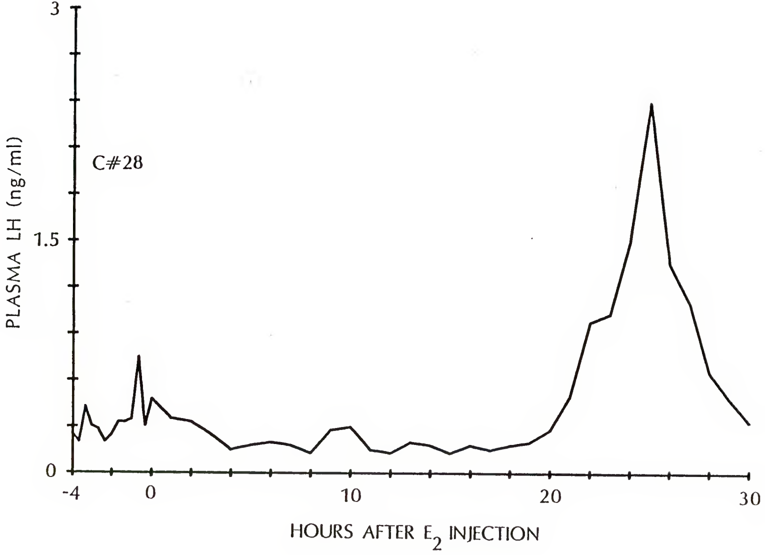

6
3
0
PLASMA LH (ng/ml)
0
15
30
HOURS AFTER E_2 INJECTION

Figure 18. Actual concentrations of plasma luteinizing hormone for one cow fed a supplement with monensin supplement and injected with E_2 at 41 days postpartum.


C#28
PLASMA LH (ng/ml)
3
1.5
0
-4
0
10
20
30
HOURS AFTER E$_2$ INJECTION

1979; Hixon et al., 1983) and in ewes (Bolt et al., 1971). The other cycling cow injected with E_2 on day 5 of the estrous cycle failed to have an increase in LH concentration within 30 h of sampling. Neither of the two cycling cows came into behavioral estrus for 11 days after E_2 injection, whereas all of the noncycling cows (no. = 12) expressed an induced behavioral estrus within 50 h after E_2 injection.

Initiation of Cyclic Activity and Pregnancy Rate Through 90 Days Postpartum

One or more signs of estrus (riding or trying to ride, standing, mucus secretion and[or] swelling of the vulva) was observed during the first detected estrus and ovulation was confirmed by a subsequent increase in concentrations of P_4. An increase in plasma P_4 above 1 ng/ml before first detected estrus was observed in 18 of the 20 cows that showed signs of estrus (table 12). Various authors have observed an increase in P_4 before cyclicity was restored in postpartum cows (Donaldson et al., 1970; Corah et al., 1974; Castenson, 1974; Webb et al., 1977; Schams et al., 1978; Berardinelli et al., 1979; Stevenson and Britt, 1979; Kesler et al., 1980; Rawlings et al., 1980; Lamming et al., 1981; Humphrey et al., 1983; Manns et al., 1983).

Naturally occurring estrus (induced estrus due to E_2 injection was not included in the calculations) was observed in 20 of the 28 experimental cows (table 13 and appendix table A-18). First postpartum estrus, during the 90-day postpartum period, tended to be slightly earlier in control GnRH cows (60 days), than in monensin GnRH (63 days), monensin E_2 (66 days) and control E_2 (69 days) cows.

TABLE 12. NUMBER OF SUCKLED BEEF COWS, FED A SUPPLEMENT WITH OR WITHOUT MONENSIN AND INJECTED WITH E_2 OR GnRH AT 41 DAYS POSTPARTUM, THAT HAD AN ELEVATION IN PLASMA PROGESTERONE BEFORE FIRST ESTRUS

Hormone	Supplement group		
	Monensin	Control	Total
E_2[b]	3(5)[a]	6(6)	9(11)
GnRH[b]	5(5)	4(4)	9(9)
Total	8(10)	10(10)	18(20)

[a] total number of cows in estrus.
[b] E_2 = estradiol-17β, GnRH = gonadotropin releasing hormone.

TABLE 13. MEAN INTERVAL (DAYS) FROM PARTURITION OR HORMONAL CHALLENGE TO FIRST ESTRUS OBSERVED IN SUCKLED BEEF COWS FED A SUPPLEMENT WITH OR WITHOUT MONENSIN AND INJECTED WITH E_2 OR GnRH AT 41 DAYS POSTPARTUM

	Monensin		Control	
	E_2	GnRH	E_2	GnRH
Before challenge	36 (1/7)[c]	33 (1/7)	38 (1/7)	38 (1/7)
Induced estrus[a]	43 ± 2.2 (6/7)	(0/7)	43.1± 1.9 (6/7)	(0/7)
Up to 77 days postpartum All cows	54.7±16.9 (3/7)	57.2±17.9 (4/7)	57.3±17.8 (3/7)	54.3±14.6 (3/7)
Excluding cows that were cycling before challenge	64 ± 7.1 (2/6)	65.3± 9.5 (3/6)	67 ± 8.5 (2/6)	62.5± 4.9 (2/6)
Up to 90 days postpartum All cows	65.8±19.4 (5/7)	63.2±20.4 (5/7)	69.6±17.9 (6.7)	60.2±16.8 (4/7)
Excluding cows that were cycling before challenge	73.2±11.5 (4/6)	70.7±13.3 (4/6)	76 ±10 (5/6)	67.6± 9.6 (3/6)

	Monensin	Control	E_2	GnRH
Cows that started cycling before hormonal challenge	34.5± 2.1 (2/14)	38 (2/14)	37 ± 1.4 (2/14)	35.5± 3.5 (2/14)
Up to 77 days postpartum[b]	64.8± 7.6 (5/12)	64.8± 6.2 (4/12)	65.5± 6.6 (4/12)	64.2± 7.3 (5/12)
Up to 90 days postpartum[b]	72 ±11.6 (8/12)	72.8±10.2 (8/12)	74.8± 8.6 (9/12)	69.4±11.1 (7/12)

TABLE 13-continued.

From challenge to 77 days postpartum	24.4± 8.8 (5/12)	21.7± 2.8 (4/12)	22.3± 5.3 (4/12)	24 ± 8.1 (5/12)
From challenge to 90 days postpartum	29 ±15.3 (8/12)	30 ± 9.5 (8/12)	32.3±10.5 (9/12)	28.7±10.9 (7/12)
Overall means	64.5±18.8 (10/14)	66 ±12.2 (10/14)	68 ±17.8 (11/14)	61.8±17.8 (9/14)

---

[a] estrus observed within 50 h after E_2 injection, all other means were calculated without including induced estrus.

[b] excluding cows that were cycling before hormonal challenge.

[c] number of cows observed in estrus over the total per group.

Monensin-treated cows showed estrus 1.5 days earlier than control cows (table 13). Noncycling cows challenged with GnRH showed estrus 5 days earlier than noncycling cows challenged with E_2 (69 vs 74 days; table 13), but more cows injected with E_2 tended to come into heat (9/12) than those injected with GnRH (7/12; table 13).

The nutritional condition of postpartum animals has an important role in the appearance of the first postpartum estrus (Wiltbank et al., 1962; Lamond, 1970; Dunn and Kaltenbach, 1980). The present data showed no effect of feeding monensin on the occurrence of the first postpartum estrus. Thus, a change in available energy due to monensin (increased propionate and decreased acetate production) did not have the beneficial effect on reducing the interval from parturition to first estrus as reported by Turner et al. (1977), Lemenager et al. (1978), Hixon et al. (1982a), Hardin and Randel (1983) and Mason and Randel (1983). The lack of an effect of monensin would agree with the second part of the experiment by Turner et al. (1977) and with Turner et al. (1980). Rawling et al. (1980) observed an interval of 59.8 days from parturition to first estrus in beef cows. Casida (1968) indicated that first estrus occurred at an average of 30 to 72 days postpartum in dairy cows and 46 to 104 days in beef cows. In the present experiment, 20 of the 28 suckled beef cows had shown estrus by 90 days postpartum with an average of 65.5 days. Kesler et al. (1980) observed that the first estrus occurred at 36 days postpartum in beef cows and GnRH injection did not affect first estrus or conception rate when compared with a saline injection.

When the intervals from hormonal challenge to first estrus were calculated for noncycling cows, within the 77-day postpartum period, five of 12 cows injected with GnRH had an estrus at 24 days postchallenge compared to 22 days for four of 12 cows injected with E_2 (table 13).

Estrous cycles considered of normal length (19 to 23 days) were observed in 6 of the 9 cows that had a second estrus within the 90-day postpartum period. The other 3 cows had estrous cycles of 15, 16 and 18 days. The mean length of the first estrous cycle was 19.2 ± 2.5 days (no. = 9). It has been shown that the first estrous cycle postpartum is shorter than the normal (Webb et al., 1980; Wettemann, 1980). However, Kesler et al. (1980) reported that the first postpartum estrous cycle of beef cows was 21 ± 1 days.

One of four cows that had expressed estrus before the hormone challenge became pregnant after being bred during the second estrus. Five of 13 cows that were bred during their first estrus became pregnant and of the 8 cows that did not conceive during the first estrus, 5 returned to estrus and 4 were rebred and became pregnant. Eighteen of the 20 cows that showed estrus were bred and 10 became pregnant (55.5%). Five of the cows that became pregnant were monensin-treated cows that conceived at an average of 76.6 ± 12.1 days and five were control cows that conceived at 69 ± 13.2 days postpartum, respectively. Five cows injected with GnRH became pregnant at 70.6 ± 11.8 days postpartum and five cows injected with E_2 became pregnant at 75 ± 14.4 days postpartum. The overall pregnancy rate was 35.7% (10/28).

DISCUSSION

Cows in the monensin-treated group had a smaller postpartum decrease in glucose concentration than control cows during the 77-day postpartum period (figure 1; $P < .05$), indicating that monensin feeding had a positive effect in maintaining elevated plasma glucose concentrations. This difference in plasma glucose concentration was probably a consequence of the changes in VFA molar percentages, i.e., increased propionate ($P < .01$) and decreased acetate ($P < .01$). This altered ruminal status would be associated with a reduction in methane production and a sparing of glucogenic amino acids due to an increase in propionate (Blaxter and Wainman, 1964; Leng et al., 1967; Reilly and Ford, 1971; Owens, 1980). The major factor responsible for the increased plasma glucose concentration was probably the increase in propionate concentration (table 5). Propionate is the major precursor of glucose (Bergman, 1973) and the percentage of glucose originating from propionate markedly increases as more propionate is absorbed (Bergman et al., 1966). Monensin itself could possibly induce an increase in glucose concentration since an i.v. injection of monensin in dogs was followed by an increase in blood glucose after 3 min (Fahim and Pressman, 1981). Furthermore, monensin can accumulate in gluconeogenic tissues of the rabbit (liver and kidney)(Fahim and Pressman, 1981) and steer (liver)(Hereberg et al., 1978). However, it is not known if monensin stimulates gluconeogenesis.

Although monensin affected ruminal VFA and plasma glucose concentrations, no significant monensin effect was observed on body weight of postpartum cows. Cows lost about 2% of their body weight from parturition to about 50 days postpartum, but by 72 to 84 days postpartum cows were heavier than on the first day after parturition (390 vs 372 kg). This indicates that cows were not at their heaviest weight at parturition and probably lost weight during gestation, which enhanced the possibility of a prolonged postpartum anestrous. A loss in body weight during late gestation indicates a negative energy balance, i.e., the need to utilize body tissue stores as a source of nutrients for the developing fetus (Dunn and Kaltenbach, 1980). These authors also observed that the interval from parturition to first estrus followed by an ovulation would be expected to increase .17 day for each kg of prepartum weight loss. Prepartum weight change is the difference between cow weight shortly after parturition and cow weight about 100 days prepartum.

The lack of a significant effect on postpartum body weight change due to monensin feeding also was observed by others (Turner et al., 1980; Walker et al., 1980a; Hixon et al., 1982b). Although Turner et al. (1980) and Walker et al. (1980a) did not observe any difference in body weight change, cows fed monensin consumed less hay indicating an increased feed efficiency. Long-term feeding of monensin may have a more marked effect on body weight than short-term feeding, since cows fed monensin for 2 years gained more weight than control cows (Turner et al., 1977). Schalles and Fleck (1979) supplemented cows on pasture during the pre- and postpartum periods with feed containing monensin and observed that cows fed monensin lost less weight. Hixon et al. (1982a) observed that pregnant heifers fed monensin were heavier than control heifers and from parturition to 120 days postpartum they lost less weight. It appears that it is necessary to start feeding monensin before parturition in order to observe a monensin effect on body weight of suckled beef cows. This would permit cows to build up tissue reserves which could be utilized during the postpartum period.

The feeding of monensin in the cows' diet had no effect on average daily weight gain of calves (.62 vs .68 kg/day) and agrees with studies of Schalles and Fleck (1979), Turner et al. (1980) and Hixon et al. (1982b). Walker et al. (1980a) observed no difference in weight gain of calves at 73 or 205 days of age whose dams were fed monensin or control rations.

Earlier observations indicated that energy level of the ration had no effect on gross uterine involution of dairy cows (Oxenreider and Wagner, 1971) or beef cows (Wiltbank et al., 1962; Dunn et al., 1969). In the present work, feeding monensin had no effect on uterine involution. Monensin feeding in this study, however, started 10 days after parturition and uterine involution was advanced already by this time, since postgravid horn diameter decreased by 50% by day 5 postpartum (Gier and Marion, 1968). A rapid decrease in uterine and cervical size was observed from days 9 to 15 postpartum in the present experiment and a plateau occurred by 30 days postpartum (figure 4). These results are in agreement with Morrow et al. (1966), Gier and Marion (1968) and Guibault (personal communication) who also detected little change in uterine involution after 30 to 40 days postpartum. The observation of Fonseca et al. (1983) and Guibault (personal communication) that uterine and cervical involution were correlated, was confirmed in the present work. Uterine and cervical diameters were highly correlated ($r = .90$; $P < .01$) indicating that cervical involution is a good indicator of uterine involution. In fact, Oltenacu et al. (1983) reported that cervical size between days 12 and 26 postpartum was a good indicator of subsequent reproductive performance in milked cows. Cows with a larger cervixes during this period had longer intervals from parturition to conception.

By 30 days postpartum, about 70% of the uteri were already in the pelvic cavity and by 70 days postpartum essentially 100% were in the pelvic canal (figure 4). The return of the uterus to the pelvic cavity was slower than that observed by Morrow et al. (1966) and Guibault (personal communication) in dairy cows, and may be due to the fact that suckled beef cows began cyclic activity later than dairy cows.

It has been suggested that uterine involution may be enhanced by an earlier restoration of postpartum, ovarian, cyclic activity in dairy cows (Mather and Melancon, 1981). The present experiment indicates that gross uterine involution in suckled beef cows was completed by 30 days postpartum. This interval is in agreement with observations by Morrow et al. (1966), Gier and Marion (1968) and Guibault (personal communication) for dairy cows. Dairy cows generally start cyclic ovarian activity earlier (i.e., 14 to 30 days postpartum) than the suckled beef cows in the present experiment, where the mean interval to ovarian cyclicity was 65 days postpartum for 20 of 28 cows that cycled. Thus, in suckled beef cows in this experiment, cyclic ovarian activity did not influence time of gross uterine involution. Oxenreider (1968) reported that uterine involution was not affected by induction of CL after parturition or by ovariectomy. However, Marion et al. (1968) pointed out that uterine involution was shortened in pluriparous dairy cows ovariectomized after parturition.

The increase in uterine size observed after E_2 injection (day 41 to day 48; figure 5) probably reflected an increase in blood supply to the uterus, followed by edema, cell proliferation and true uterine growth due to high estrogen concentration (Asdell et al., 1949; Clark et al., 1977). It has been shown that injection of estrogen in physiological dosages delayed uterine involution, probably by increasing uterine wall thickness (Marion et al., 1968). Britt et al. (1974) observed that cows implanted 14 days postpartum with GnRH had an enhanced rate of uterine involution, which was later confirmed by Fernandes et al. (1978b). This could also contribute to the differences in uterine size observed in the present experiment after hormonal challenge (E_2 vs GnRH). Mean uterine size of GnRH-treated cows between days 42 and 48 was smaller (figure 5) than the means observed before 42 days or after 48 days postpartum. A small reduction in size at the time E_2 increased uterine size could enhance the difference between the two hormonal groups. However, a group without hormone treatment was not used in the present experiment. Britt et al. (1974) indicated that the increase in plasma P_4 due to GnRH implant could be responsible for the enhanced uterine involution observed in dairy cows as compared to cows receiving a saline implant. In the present study, GnRH caused an increase in plasma P_4 ($>$ 1 ng/ml) in only 6 of 12 cows; thus, there is the possibility of a direct GnRH effect on uterine size.

The observation that monensin-treated cows had greater ovarian activity (figures 6 to 10) than control cows is in agreement with Wiltbank et al. (1964), who noticed a greater ovarian activity in cows fed high energy levels. Howland et al. (1966) observed that ewes fed grain had larger ovarian follicles and more follicular fluid than ewes not fed grain. Prepuberal heifers fed monensin for 15 days had larger ovaries than the controls (Bushmich et al., 1980). Other authors were unable to detect differences in ovarian activity due to feeding different levels of energy (Spitzer et al., 1978; Lishman et al., 1979).

The difference in ovarian volume that occurred early after initiating monensin feeding (3 to 6 days; figure 8) indicated that monensin had a direct effect on the ovary. An indirect effect, via change in VFA concentrations or an altered metabolite response (i.e., glucose), probably would take longer. Fahim and Pressman (1981) pointed out that monensin would pass easily through the gut into the blood and be taken up preferentially by tissues with a high rate of metabolism. They also reported that the .05 ppm residue permitted in cattle and chicken tissues, would be achieved and exceeded in even casual nonchronic feeding experiments. No reference was found relating a possible direct action of monensin on the ovaries. The number of follicles greater than 5 mm in monensin-treated cows increased at about 10 to 15 days after monensin feeding started, i.e., 20 to 25 days postpartum (figure 6). A difference of 10 to 15 days from the increase in ovarian volume (at 3 to 6 days after monensin feeding started) and the initiation of follicular growth detectable by palpation suggests a latent period of 10-15 days for follicular recruitment. During the estrous cycle, a follicle takes 27 days to grow from 0.13 mm to 0.67 mm and 14.2 days from 0.68 mm to 8.5 mm (Lussier et al., 1983).

Cows injected with GnRH developed more follicles than E_2-treated cows and monensin feeding had an additive effect, since cows fed monensin and challenged with GnRH developed more follicles than control cows (figure 7). Prepuberal heifers fed monensin and injected with FSH-P had more ovulation sites, CL numbers, follicular fluid and total ovarian weight than control hiefers injected with FSH-P (Bushmich et al., 1980). Cycling heifers fed monensin and injected with FSH-P had heavier CL and higher serum P_4 concentration from day 5 to 11 of the estrous cycle than control heifers (Harrison et al., 1982). Allen and Lamming (1961), using ewes, and Lamond (1970), using beef heifers, observed that the nutritional effect on follicular development was more pronounced after PMSG injection. This seems to be the situation in the present experiment where cows fed monensin developed more follicles and had greater ovarian volume after injection of GnRH or E_2 than did control cows after hormonal challenge (figures 7 and 10). Since the predominant effect of the ionophores (i.e., monensin) is to elevate cell Na and promote K exodus (Austic and Smith, 1980), the possibility of change in excitability of cell membrane exists if effective monensin levels get into specific tissues (Bergen and Bates, 1983, in press). Thus, a direct effect of monensin on the cells of the ovary, if present, may induce change in cell sensitivity. A change in hormone receptor population, for example, could increase the sensitivity to the same concentration of hormone. The basal LH concentrations did not differ in the present experiment between monensin and control cows when measured at 41 days postpartum for 4 h at 20 min intervals or every 3 days during the first 38 days postpartum. However, the number of follicles and the ovarian volume were greater in monensin-treated than in control cows during the first 38 days postpartum (appendix tables A-5 and A-7), indicating a greater stimulation of the ovaries of monensin cows by the same basal LH concentrations. Monensin-treated cows released less LH than control cows (figure 16; table 11) in response to an injection of GnRH. Monensin-treated cows injected with GnRH had more follicles, however, than control cows injected with GnRH, indicating that the sensitivity of the ovaries to gonadotropin may have been affected by feeding monensin.

Glucose blood concentrations increased as energy level of feed increased (Howland et al., 1966; Oxenreider and Wagner, 1971; Downie and Gelman, 1976) and an increase in blood glucose could stimulate the hypothalamic centers controlling gonadotropin secretions and induce greater ovarian activity (Howland et al., 1966). McClure (1972) suggested that low blood glucose could affect the secretion of GnRH by depressing the nervous system activity. In an earlier study (McClure, 1968), insulin injection from days 17 to 20 of the estrous cycle of cows either inhibited estrus or reduced pregnancy rate.

It has been shown that LH concentrations were decreased by reducing the nutritional level of female ruminants (Apgar et al., 1975; Jordan and Swanson, 1979; Haresign, 1981; Gauthier et al., 1983), or increased (Gombe and Hansel, 1973). Thus, a low level of nutrition may decrease LH concentrations probably by affecting ability of the hypothalamus to release and (or) produce GnRH. Injections of GnRH induced a higher LH surge in heifers and ovariectomized cows

(Beals et al., 1978), in intact cows (Lishman et al., 1979; Mason and Randel, 1983; present study) and in ewes (Haresign, 1981) fed low levels of energy. Thus, nutritional inhibition is not likely to be at the pituitary level. Others observed no difference in the GnRH induced LH surge in cows fed different levels of nutrition (Entwistle and Oga, 1975; Cummins et al., 1975). An increase in energy level was related to an increase in LH response due to GnRH in prepuberal heifers fed monensin (Randel and Rhodes, 1980) and in prepuberal heifers infused with propionate (Rutter et al., 1983). The LH response to estrogen injection was greater in postpartum beef heifers fed 40% less energy than in the controls (Echternkamp et al., 1982) and in prepuberal heifers fed 200 mg of monensin (Randel et al., 1982).

Although there are contradictory results in terms of LH response due to GnRH, the majority of the results indicate that an increase in the nutritional level is responsible for an increase in basal LH. It is reasonable to assume that a greater ovarian stimulation would occur due to an increase in basal LH concentration. In the present work, the LH concentration tended to increase with time postpartum. However, basal LH concentrations measured for 4 h at about 41 days postpartum did not differ between monensin-treated and control cows (.24 vs .23 ng/ml). Basal LH concentrations of monensin-treated cows were more variable probably due to the effect of increased energy availability since monensin-treated cows had a smaller ($P < .05$) decrease in glucose concentration (figure 1) than did control cows during the postpartum period. A higher glucose concentration may stimulate the hypothalamic centers to release more GnRH which would stimulate the pituitary to release LH in a pulsatile manner. Various authors have observed an increase in frequency and amplitude of LH secretion before ovarian cyclicity was restored in postpartum cows (Rawlings et al., 1980; Humphrey et al., 1983). Tonic levels of gonadotropins induce follicular maturation, consisting of granulosa and theca cell proliferation and steroidogenesis (Dunn et al., 1979). Thus, a greater variability in LH concentrations at day 41 postpartum due to monensin feeding could also be responsible for a greater stimulation of the ovaries observed in the present experiment. However, this difference in variability was not assessed at the time follicular development was first noted, i.e., at 20 to 25 days postpartum.

Mean concentration of plasma P_4, although increasing slightly, remained below 1 ng/ml during the first 38 days postpartum. This is indicative that no luteal tissue was producing P_4 and is in agreement with Webb et al. (1977). Entwistle and Oga (1977), Goodale et al. (1978) and Webb et al. (1980) also did not observe a significant increase in P_4 concentrations in anestrous cows. In the present experiment, an increase in LH concentration, number of follicles and ovarian volume was observed at the same time that P_4 was increasing during the first 38 days postpartum.

The trend of plasma LH to increase during the postpartum period was observed by others (Edgerton and Hafs, 1973; Humphrey et al., 1976; 1983; Kesler et al., 1977; 1980; Fernandes et al., 1978a; Goodale et al., 1978; Stevenson and Britt, 1979). Echternkamp (1978) suggested that the increase in plasma LH concentrations during the postpartum period could be due to an increase in pituitary content.

Saiduddin et al. (1968) and Wagner et al. (1969) observed that pituitary LH content increased during the postpartum period. Positive estrogen feedback on the neuroendocrine center also has been suggested to cause an increase in circulating LH concentrations (Stevenson and Britt, 1979). The return to cyclic ovarian activity in postpartum cows is preceded by a constant increase in pulsatile releases (Humphrey et al., 1976) and in basal concentration of LH (Lamming et al., 1981). Increases frequency and magnitude of peaks of LH, before first estrus, have been suggested as being responsible for the increase in basal LH concentrations (Rawlings et al., 1980; Humphrey et al., 1983).

The LH response to GnRH is restored by 15 to 20 days postpartum in dairy cows (Fernandes et al., 1978a; Foster et al., 1980) and from 15 to 28 days in suckled beef cows (Kesler et al., 1980; Irvin et al., 1981) and, since the GnRH challenge in this study was done at 41 ± 1.5 days postpartum in suckled beef cows, no inhibitory effect of the early postpartum interval would be expected. Kesler et al. (1980) reported that all suckled beef cows injected with GnRH had an LH surge while only one injected with saline had an LH rise.

The fact that control cows released more LH than monensin-treated cows (figure 16; table 11) in response to one GnRH injection (100 ug) is in agreement with the study of Mason and Randel (1983), where suckled beef cows in a control group released more LH than monensin-treated cows.

In the present experiment, variation in energy available to the cows was due to monensin feeding which theoretically can increase the amount of available metabolizable energy by up to 20% (Rowe et al., 1981). Richardson et al. (1976) estimated that monensin could increase the availability of energy by 6%. No difference in basal LH concentrations measured at 41 days postpartum was observed in the present experiment (table 6). Gauthier et al. (1983) fed 33% less net energy to suckled beef cows and observed a lower basal LH in underfed cows. They also observed that mean daily weight gain from 0 to 45 days postpartum was correlated with basal LH concentrations on day 30 postpartum ($r = .58$; $P < .01$; no. = 19). This agrees with Echternkamp et al. (1982), who also reported a high correlation ($r = .75$) between average daily weight gain and basal LH concentrations. Higher ($P < .01$) concentrations of LH were measured in suckled beef heifers fed the high energy ration. In the present experiment, the mean basal LH concentration prior to GnRH injection was correlated with the mean LH concentration after GnRH injection ($r = .56$; $P < .05$) and with the peak LH value ($r = .52$; $P < .06$), indicating that cows with a higher basal LH concentration released more LH after a GnRH injection. This would agree with the "self priming" effect, i.e., previous exposure of the pituitary to LHRH increases the quantity of LH released by a subsequent LHRH challenge (Kesner et al., 1981).

Beal et al. (1978) reported that heifers and ovariectomized cows fed 57 and 36% less net energy, respectively, released more LH than the controls to one injection of GnRH. However, no difference was observed in LH response of cycling cows fed 36% less net energy than controls cows. Ovariectomized cows were free from LH inhibitory factors from ovarian origin (e.g., P_4, estradiol and inhibin) and basal LH concentrations were increased compared to preovariectomy values. This would allow an increase in sensitivity of the pituitary due to the "self priming" effect of LHRH (Kesner et al., 1981). It has been suggested that the higher response to GnRH injection in underfed animals could be due to an increase in pituitary LH content in association with a decrease of LH secretion in the plasma, i.e., the amount of releasable stored LH in the pituitary may be higher in underfed animals since the pituitary is secreting less LH (Gauthier and Mauleon, 1983). This would agree with the fact that underfed cows (Gauthier et al., 1983) and ewes (Haresign, 1981) had lower basal LH concentration and released more LH after GnRH injection. Thus, underfed, ovariectomized cows could have an increased sensitivity due to a lack of ovarian inhibitors and a bigger releasable pool of LH in the pituitary. This could be responsible for the difference observed in LH response due to an injection of GnRH in ovariectomized cows fed low levels of energy compared to cycling cows (Beal et al., 1978).

Gauthier et al. (1983) concluded that the reduction in gonadotropin secretion in underfed cows suggests a direct effect of nutritional deprivation on hypothalamic function rather than a defect in pituitary physiology per se, since it has been shown that the delayed first postpartum ovulation induced by underfeeding is not due to the failure of the pituitary to respond to GnRH with an LH surge but due to low chronic gonadotropin concentrations.

No complete LH surge due to E_2 (10 mg; i.m.) was observed in any of the 12 noncycling suckled beef cows within 30 h after E_2 injection and feeding monensin had no effect on LH concentration. Echternkamp et al. (1982) observed that suckled beef heifers fed low levels of energy, 30% of the requirements during pregnancy and 40%

during lactation, released less LH due to an injection of 10 mg of estradiol benzoate at 14 and 28 days postpartum (experiment one) and at 14, 32, 50 and 74 days postpartum (experiment two) than heifers fed a high energy ration. Hardin and Randel (1983) observed no difference in the amount of LH released after an injection of 0, 1, 2 or 4 mg of E_2 between suckled beef cows fed monensin or a control ration for 20 days. They also showed that cows fed monensin had a delay in the LH peak (30 h) compared to control cows (18 h) after the injection of 4 mg of E_2.

The responsiveness to estrogen injection, in terms of LH release, is restored by 2 to 3 weeks postpartum in suckled beef cows (Nancarrow et al., 1977; Short et al., 1979; Echternkamp et al., 1982) or up to 7 weeks (Radford et al., 1978; Smith et al., 1981). In the present experiment, the LH surge induced by E_2 started at about 24 h in 7 cows and only one cow had a complete peak within the 30 h (figure 18). The observation of Smith et al. (1981) that suckled cows did not have an LH response during the first 6 weeks postpartum may not be correct because they did not bleed long enough (24 h), as results in the present experiment indicated. Various authors reported that suckling had no significant effect on LH release due to an injection of 10 to 20 mg of E_2 (Short et al., 1979; Forrest et al., 1980). Forrest et al. (1980) observed that suckling depressed the duration of the LH surge indicating some blockage due to suckling stimuli. Randel et al. (1981) reported that suckling impaired hypothalamic-pituitary response to low E_2 challenge dose (< 1 mg) and elicited changes in timing parameters in response to high E_2 dosage. They also observed that nonsuckled cows completed the LH surge earlier (27.14 vs 32 h;

$P < .05$) than cows suckled every 6 h for 30 min. Acosta et al. (1983) suggested that suckling increased the sensitivity of the hypothalamus to the negative feedback of estrogen. Cows were suckled ad libitum in the present experiment, and due to the confinement of the cow and calf in one stanchion where hay and water were not supplied ad libitum an increase in suckling intensity was probable. This could delay the LH surge more than the delay observed by Randel et al. (1981) in the nonsuckled cow.

Gauthier et al. (1983) fed 33% less net energy to suckled beef cows and observed a decrease in basal LH concentrations. Observing the different levels of energy fed and physiological status of the animals in various studies (Beal et al., 1978; Lishman et al., 1979; Echternkamp et al., 1982), it can be observed that mature cows or cycling heifers showed differences in LH response to GnRH or estrogen challenge when receiving 80% or less of the energy fed to the control animals (100% of the recommended levels or above).

Prepuberal heifers fed a supplement with monensin released more LH following two GnRH injections 6 h apart (Randel and Rhodes, 1980) or one E_2 injection after 14 days on monensin (Randel et al., 1982). Prepuberal heifers may be more sensitive to change due to monensin feeding than mature animals and also their neuroendocrine system is not completely developed, i.e., not used to the hormonal variations of the normal estrous cycle.

This would suggest that cycling heifers or mature cows probably can adjust better to small variations in amount of energy fed than prepuberal heifers. Also, if monensin can increase the sensitivity of the target cell via changes in ion concentration in the cell, the possibility of a different response to a hormonal challenge exists mainly in prepuberal heifers which are not used to large variations in hormonal concentrations, e.g., P_4. However, LH concentrations in heifers are more variable during the prepuberal period than after cyclicity starts, probably because of the lack of the inhibitory effect of P_4 (Gonzalez-Padilla et al., 1975). They also observed that the 2 months preceding the onset of puberty is characterized by a lack of the cyclic pattern of release of LH, which appears gradually.

The increase in P_4 concentrations observed from 39 to 77 days postpartum was related to ovulations and is in agreement with Morrow et al. (1966), Marion and Gier (1968), Edgerton and Hafs (1973) and Fernandes et al. (1978a).

Although ovarian activity (more follicles and larger ovaries) was affected by feeding monensin and monensin-treated cows maintained a higher concentration of glucose during the postpartum period, no effect of feeding monensin was observed on P_4 concentrations. Apgar et al. (1975), Beal et al. (1978) and Spitzer et al. (1978) reported that P_4 concentrations in Holstein heifers, beef cows and beef heifers, respectively, were not affected by levels of nutrition. However, Hill et al. (1970), Gombe and Hansel (1973) and Beal et al. (1978) observed that underfed heifers had lower concentrations of P_4 than controls. Underfed animals have a metabolism that differs from that of the well-fed animal. The underfed animal mobilizes body reserves for maintenance and(or) production, while the well fed animal will deposit body tissues. This difference may be due to a decreased availability of substrate for P_4 production or due to a greater turnover in tissue thus increasing the clearance rate of P_4, and consequently, decreasing the P_4 concentration in the circulation of underfed animals. Corpora lutea of underfed heifers produced less P_4 than CL of control heifers when incubated with different levels of LH (Apgar et al., 1975). This could indicate a difference in the metabolism of the CL between the two treatments or a difference in sensitivity of the luteal cells to LH.

The LH concentrations decreased when low levels of nutrition were fed to female cattle (Apgar et al., 1975; Beal et al., 1978; Gombe and Hansel, 1973; Dunn et al., 1974); however, there still are contradictions on nutritional level affecting P_4 concentrations. This shows the difficulty of relating a P_4 plasma concentration to the functional CL.

No differences in concentrations of P_4 and LH (measured every 3 days for 38 days or every 20 min for 4 h) due to feeding monensin were detected in this study. However, the basal LH concentrations (measured every 20 min for 4 h) were more variable in monensin-treated than control cows at about 41 days postpartum or after 31 days of monensin feeding. By this time, monensin-treated cows had more follicles, larger ovaries and higher glucose concentrations than control cows.

Postpartum day trends for cumulative P_4 during the 77 days postpartum (table 3; $P < .01$) and after hormonal challenge (39 to 77 days postpartum; figure 13; $P < .01$) indicate that GnRH injection could increase ovarian activity more than E_2 injection. This was confirmed by the greater follicular activity induced by GnRH treatment (figure 7).

The hormone injections (GnRH or E_2) induced a transitory increase in P_4 concentrations that lasted for 3 to 6 days in 50% of the 24 anestrous cows. Such a response is suggestive of a luteinization of some ovarian structures such as follicles. Although a complete LH peak in plasma of suckled anestrous cows could not be detected within 30 h of E_2 injection (sampling stopped at 30 h) it appeared that a preovulatory surge of LH did occur in at least 50% of the E_2 injected cows. It was noted that 6 of 12 noncycling cows had an initial LH rise above basal concentrations (LH value at 30 h ranged from 2.1 to 25.6 ng/ml) and concentrations of P_4 increased for up to 6 days in 50% of the anestrous cows. The P_4 changes are indicative of a possible luteinization of ovarian follicles or formation of a CL with a short life span.

Only one GnRH treated cow appeared to have ovulated and had an estrous cycle of normal duration based on the profile of P_4 concentrations. Five other cows had an increase in P_4 concentrations after GnRH injection that lasted for 3 to 6 days. An increase in P_4 and (or) ovulation due to a GnRH injection in cattle has been observed by others (Britt et al., 1974; 1975; Kesler et al., 1977; 1980; Webb et al., 1977; Fonseca et al., 1980; Troxel et al., 1980; Wettemann et al., 1982).

Although dairy cows ovulated in response to one GnRH implant at 14 days postpartum (Britt et al., 1974; 100% ovulation), one injection of GnRH from 1 to 19 days postpartum in diary cows (Kesler et al., 1978) and in suckled beef cows between 20 and 45 days postpartum was followed less frequently by a palpable CL, probably because an implant releases GnRH longer and more constantly than one

GnRH injection. However, a transient increase in P_4 concentration occurred in suckled beef cows for 3 to 9 days after one injection of GnRH (Britt et al., 1975; Webb et al., 1977; Kesler et al., 1980; Wettemann et al., 1982; present work). Troxel et al. (1980) pointed out that calf removal for 24 h prior to GnRH injection improved the ovulation response to GnRH. In contrast, Fonseca et al. (1980) reported that calf removal did not increase ovulatory response to GnRH. Removal of the suckling stimulus led to an increase in pulsatile LH release (Walters et al., 1982a). In the present experiment, calves stayed with their mothers before, during and after the bleeding. Calves were confined with their mothers in individual stanchions during the bleeding period. This was done so that cows would be more docile during the bleeding period. It was observed that the calves seemed to suckle more frequently during the bleeding period possibly because hay and water were not offered ad libitum.

Webb et al. (1977) observed that treatment of suckled cows with one LHRH injection early after parturition was not able to initiate ovarian cyclicity although a transient increase in P_4 concentration was observed. However, when a second LHRH injection was given after the transitory increase in P_4, it appeared to initiate normal cyclic activity. The CL or luteinized structure subsequent to one GnRH injection in most cases had a shorter life span than a CL of a normal estrous cycle (Lishman et al., 1979; Kesler et al., 1980; Fonseca et al., 1980).

Cows fed monensin had greater follicular activity before GnRH injection; however, this did not result in a greater number of ovulations or increased luteal activity after GnRH injection, since the control cows injected with GnRH secreted more P_4 than cows fed monensin and injected with GnRH (figure 13). However, a group without GnRH or E_2 injection was not evaluated in this experiment. Britt et al. (1974) observed that 100% of dairy cows implanted with a capsule that contained GnRH ovulated while controls did not. Kesler et al. (1980) reported that 50% of suckled beef cows injected with GnRH ovulated compared to 0% in the control group. The fact that cows injected with E_2 secreted less P_4 than cows injected with GnRH may be due to a less consistent and precise response in release of LH due to E_2 injection. Clark et al. (1979) observed that subcutaneous implantation of capsules containing crystalline 17-estradiol for 48 h on day 6 of the menstrual cycle of Rhesus monkeys induced an LH surge. However, it also induced a rapid regression of the follicle destined to ovulate. Such an effect could have influenced the ovarian response of the cows injected with E_2. Cows injected with E_2 developed fewer follicles than cows injected with GnRH (figure 7; appendix table A-8). It was observed that one control cow injected with E_2 on day 5 of the estrous cycle had a shorter estrous cycle (16 days) than another cow in the monensin-fed group injected with E_2 on day 4 of the estrous cycle. The latter cow had a small peak of LH (2.4 ng/ml) 25 h after E_2 injection (figure 18). Bolt et al. (1971) observed that estrogen had a luteolytic effect in intact ewes injected on day 10 of the cycle but not in hysterectomized ewes and not when administered on day 3 of the estrous cycle.

There were no appreciable differences in time when the first natural estrus occurred in the suckled beef cows fed a supplement with or without monensin (64.5 vs 66 days postpartum, respectively). Cows injected with GnRH tended to show spontaneous estrus earlier than cows injected with E_2 (61.8 vs 68 days postpartum; table 13). However, the period from hormonal challenge to noninduced estrus was about the same for control cows injected with GnRH, cows fed monensin and injected with GnRH, cows fed monensin and injected with E_2, and control cows injected with E_2 (28.7, 30, 29 and 32.3 days postchallenge, respectively; table 13).

Induced estrus was observed in all noncycling cows within 50 h after injection of E_2 (12/12) but in none of the noncycling cows injected with GnRH. However, one cow injected with GnRH probably ovulated without showing estrus since she had a 20-day interval from GnRH treatment to first observed estrus with P_4 concentrations above 1 ng for 13 days. Five other cows injected with GnRH and six cows injected with E_2 had increases in P_4 above 1 ng/ml for up to 6 days but no CL was detected via rectal palpation in any of these during that period.

The observation that GnRH injection did not induce estrous behavior is in agreement with Britt et al. (1974), where all noncycling dairy cows implanted with GnRH on day 14 postpartum failed to show estrus. However, all of them ovulated within 24 h after the start of GnRH treatment. The first ovulation occurred at 14.4 and 23.6 days postpartum for GnRH and saline treated cows, respectively, while first postpartum estrus was observed at 41 and 37.5 days for the GnRH and saline groups, respectively. The observation of 100% ovulation due to GnRH implant (Britt et al., 1974) may be because an implant of GnRH will release a more constant amount of LH for a longer period of time. This may induce a greater period of elevated LH and ovarian response compared to a single injection.

SUMMARY AND CONCLUSIONS

Suckled beef cows were supplemented individually from parturition to day 90 postpartum with a concentrate mixture of corn and soybean meal (16% CP and 85% TDN) according to their metabolic weight. All cows received the same concentrate from parturition to day 9 postpartum. From day 10 to day 90 postpartum or until diagnosed pregnant via embryo recovery 17 to 18 days postbreeding, the cows (no. = 28) were divided into two groups and supplemented with the concentrate without or with addition of monensin (200 mg per cow daily). Cows also received about 8.6 kg of coastal bermudagrass hay (4.5% CP) per day on a pasture of bahiagrass and white clover. The concentrate and hay supplied the minimum NRC (1976) recommendations for protein and energy of suckled beef cows of average milk production. The calving season lasted from January 2 to February 14, 1983. All cows were bred previously to Brahman bulls. Cows and calves were weighed and a blood sample taken from the dam within 24 h of birth. Cows were bled via the jugular vein every third day before feeding the supplement. Plasma was obtained by centrifugation (4 C), stored at -15 C and analyzed for P_4, LH and glucose. Rectal palpation of the uterus (location, tonus and diameter), cervix (diameter), and ovarian dimensions (length, height, width) and structures (follicles, CL) was performed at 3-day intervals starting about day 9 postpartum. A rumen fluid sample was collected at approximately 47 days postpartum, 6 to 8 h after the cows were fed the concentrate supplement.

At 41 days postpartum, cows fed supplement (with or without monensin) were subdivided into two subgroups where 14 cows (7 from monensin and 7 from control) were challenged with GnRH (100 ug in 2 ml sterile solution, i.v.) and the others (no. = 14) were challenged with E_2 (10 mg in sesame oil, i.m.). All cows were bled, via a catheter, from the jugular vein for 4 h at 20 min intervals before hormonal challenge to determine the basal plasma LH concentration. After hormonal challenge cows injected with GnRH were bled at 5, 10, 20, 30, 40, 50, 60, 70, 80, 90, 120, 150, 180, 210, 240, 270, 300, 330, 360 min, whereas cows injected with E_2 were bled hourly for 30 h. Plasma was obtained and stored at -15 C for LH determination.

Cows fed monensin maintained a higher plasma glucose concentration than control cows. This probably was associated with the higher ruminal propionate ($P < .01$) and lower acetate molar percent ($P < .01$) and acetate: propionate ratio ($P < .01$) observed in the monensin group. The differences in glucose concentrations were more pronounced during the second half of the 77-day postpartum period (70.2 vs 68.2 mg/dl) than during the first half (72.4 vs 72.1 mg/dl) for monensin-treated and control cows, respectively.

Gross involution of the cervix and uterus were highly correlated ($r = .90$; $P < .01$) and were completed by 30 days postpartum with no difference detected between monensin-treated and control cows. Therefore, gross uterine involution was not a limiting factor for restoration of the cyclic activity that occurred on the average of 65 days postpartum in 20 of the 28 cows.

Cows fed monensin had larger ($P < .1$) ovaries than controls. The ovarian volume started to differentiate 3 to 6 days after the start of monensin feeding. This would suggest a direct effect of monensin on the ovaries. Cows fed monensin also had more ($P < .1$) follicles than control cows, which could influence ovarian volume, since the correlation between ovarian volume and number of follicles was significant (r = .33).

A simultaneous increase in LH and P_4 concentrations during the first 38 days postpartum probably indicated that LH stimulated P_4 secretion from ovarian follicles. More follicles were detected in monensin treated cows, indicating that they should have a higher rate of gonadotropin secretion (LH and FSH) which is necessary for follicular growth unless monensin directly stimulated follicular growth or influenced tissue responsiveness to endogenous gonadotropin concentration. A high LH secretion is supported partially by the greater variation in LH concentrations of monensin-treated cows in an acute sampling sequence just prior to challenge with either E_2 or GnRH at about 41 days postpartum or 31 days after monensin feeding began.

An increase in P_4 concentration above 1 ng/ml was observed prior to first estrus in 18 of the 20 cows that showed estrus within 90 days postpartum. Increased incidence of estrus and ovulation occurred after 38 days postpartum and this was reflected in the mean P_4 concentrations which tended to be higher than 1 ng/ml. Progesterone concentrations were not affected by the dietary supplement. Fifty percent of the cows of each hormonal treatment group (GnRH and E_2) had an increase in P_4 above 1 ng/ml that lasted up to 6 days except one cow that had a P_4 concentration above 1 ng/ml for 13 days. This increase in P_4 after challenge probably was related to an increase in LH due to GnRH or E_2 injection with subsequent luteinization of ovarian tissue or ovulation. However, no CL was palpated during this period. Control cows released more ($P < .01$) LH than monensin-treated cows in response to GnRH. GnRH challenged cows also secreted more P_4 than E_2 challenged cows probably due to greater LH response to GnRH that subsequently induced increased ovarian activity with more follicles and larger ovaries.

Luteinizing hormone released following GnRH was less for the monensin-treated group ($P < .01$) probably due to a reduced releasable pool of LH associated with a greater and more variable rate of LH secretion at the time of GnRH challenge. Therefore, ovarian and hormonal status (increased variability in basal LH) could indicate that monensin-treated cows were ahead of the control cows relative to restoration of normal cyclicity. Rawlings et al. (1980), Lamming et al. (1981) and Humphrey et al. (1983) observed an increase in frequency and magnitude of LH peaks during the postpartum period before cyclicity was restored. The larger number of follicles observed in monensin cows could be responsible for the greater variability in basal LH via an increased production of estrogen. However, estradiol was not measured in the present experiment. Higher glucose concentrations could be also responsible for a more variable basal LH concentration by accelerating the return to a pulsatile pattern of LH that is induced by episodic release of GnRH from the hypothalamus. The mean basal LH concentration prior to GnRH challenge was correlated with mean LH release during a 6-h period after GnRH injection ($r = .56$; $P < .05$) and with the LH peak concentration ($r = .52$; $P < .06$). The LH response of the anestrous suckled beef cows to the E_2 injection took longer than expected probably due to the increased suckling activity during the bleeding period.

There were no significant differences in the interval from calving to first estrus due to monensin feeding (64.5 days [10/14] vs 66 days [10/14] postpartum, respectively) or due to GnRH or E_2 injection (62 days [9/14] vs 68 days [11/14] postpartum, respectively). Ten cows became pregnant (35.7%) within the 90-day postpartum period (five cows from the monensin group and five from the control group). Five cows became pregnant in each of the GnRH and E_2 groups.

From the present data, it was be concluded that by feeding monensin to suckled beef cows plasma glucose concentrations after parturition were changed ($P < .05$) and both the number of follicles and ovarian size were increased ($P < .1$). However, this stimulatory effect of monensin on glucose metabolism and follicle growth was not translated into an increase in frequency of ovulation or cyclic activity. Apparently, the suckling stimulus inhibited any selective advantage of monensin supplementation. Monensin-treated cows did have more variable concentrations of LH at 41 days postpartum with a difference in LH response to GnRH (monensin < control). These responses were interpreted as representing a greater basal secretion of LH in the monensin group. There was no effect of feeding monensin on pregnancy rate or on the interval from parturition to first estrus.

The results of this experiment agree with the observations of Walker et al. (1980a), Hixon et al. (1982b) and Mason and Randel (1983) who did not observe differences in cow weight due to feeding monensin. The results, however, disagree with Turner et al. (1977), who observed that cows fed monensin gained more ($P < .05$) weight than controls in two trials of 1 year each, and Shalles and Fleck (1979), who observed that cows fed 200 mg daily of monensin lost less weight ($P < .01$) than control cows or cows fed 400 mg of monensin per day.

In the present experiment, there was no effect of monensin on the interval from parturition to first estrus in the 20 cows which showed estrus. This is in agreement with Walker et al. (1980a), but does not agree with the results reported by Turner et al. (1977; 1980), Hardin and Randel (1983) and Mason and Randel (1983), who observed a shorter interval from parturition to first estrus in cows fed monensin. Turner et al. (1977; 1980) observed differences in VFA concentrations in cows fed monensin as compared with the control cows. The same result was observed in this study. Hixon et al. (1982b) observed no difference in P_4 concentrations due to feeding monensin to postpartum cows, a finding which agrees with that of this study. Hardin and Randel (1983) reported no effect of monensin on LH response due to E_2 injection. Basal LH was not affected by feeding monensin (Mason and Randel, 1983); but these authors did observe that cows fed monensin released less ($P < .005$) LH, in response to one injection of GnRH, than control cows. The same was confirmed in the present study.

Further studies of the effects of monensin on reproduction should consider the possibility of direct and(or) indirect effects of monensin on the reproductive system. Monensin could be supplied in the feed or via infusion into the abomasum and(or) jugular vein. Histological studies of cells of the hypothalamus, pituitary, ovaries and uterus should be carried out. An attempt also should be made to determine the number of receptors for GnRH in the pituitary and for LH in the ovaries at different times during the reproductive processes.

In vivo studies of the LH response to GnRH injection should consider two or more injections of GnRH at short time intervals. Also, an in vitro study should be made of the pituitary function of animals treated with monensin in terms of ability to secrete LH and to respond to GnRH. In vitro studies, however, may not be a good indicator of what happens in vivo since monensin can be metabolized in the animal and it will be very difficult to maintain the same effect of monensin on the cell membrane. The effect of monensin on the cell membrane may be reversible, and a change of ion and monensin concentration from the in vivo to in vitro environment may change completely the response of the pituitary cells.

APPENDIX

Figure A-1. Inhibition curves of iodinated bovine LH (USDA-bLH-I-1) by masses of LH standard (USDA-bLH-B-5; A) and by a plasma sample assayed at six different volumes (.25, .50, .100, .150, .200 and .300 ml; B).

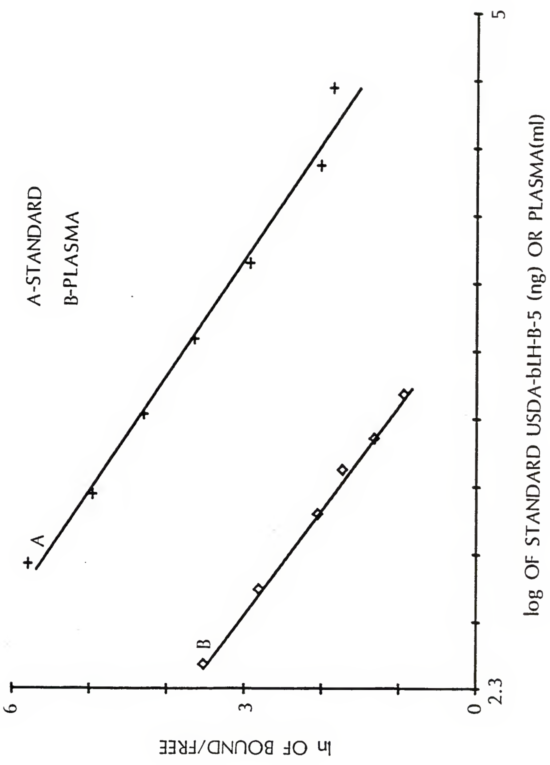

A-STANDARD
B-PLASMA
A
B
ln OF BOUND/FREE
6
3
0
2.3
5
log OF STANDARD USDA-bLH-B-5 (ng) OR PLASMA(ml)

Figure A-2. Least squares regressions of LH measured in 100 (A) or 200 ul (B) of a plasma containing differents concentrations (0, .25, .5, 1, 4, 6, 8, 10 and 12 ng/ml) of LH standard (USA-bLH-B-5).

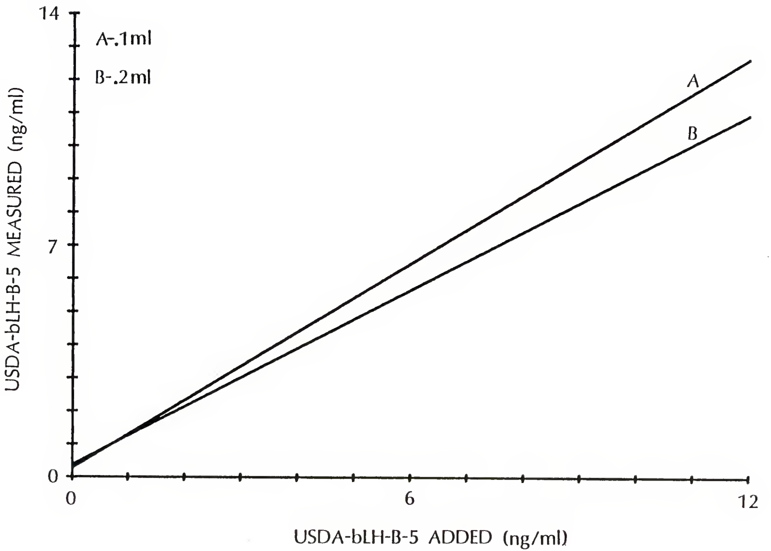

14
7
0
A-.1ml
B-.2ml
A
B
0
6
12
USDA-bLH-B-5 MEASURED (ng/ml)
USDA-bLH-B-5 ADDED (ng/ml)

Figure A-3. Progesterone concentrations measured at 3-day intervals in cows (C) fed a supplement with or without monensin and injected with GnRH or E_2 at 41 days postpartum. These are the values from the 13 cows that showed estrus (H) within 77 days postpartum.

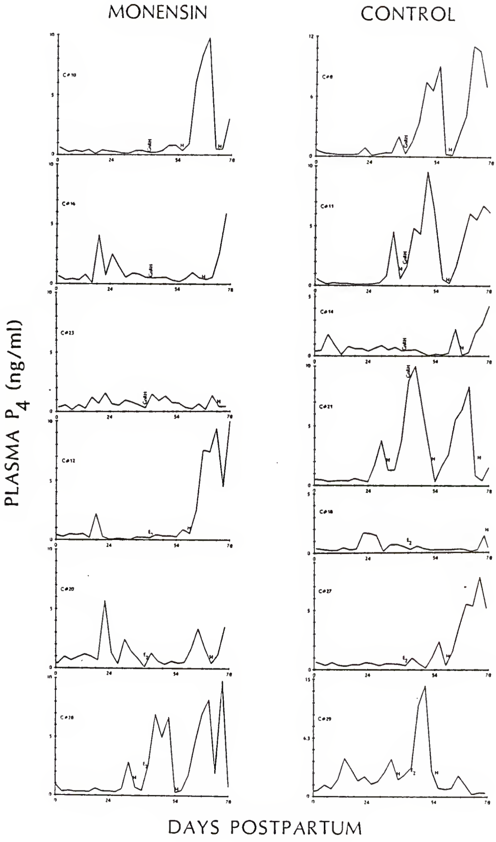

MONENSIN
CONTROL
PLASMA P_4 (ng/ml)
DAYS POSTPARTUM
C#10
C#16
C#23
C#12
C#20
C#28
C#8
C#11
C#14
C#21
C#18
C#27
C#29
GnRH
H
E_2
0
5
10
12
6
15
6.3
24
54
78

TABLE A-1. AVERAGE DAILY AMOUNT (ADA; kg) OF SUPPLEMENT WITH OR WITHOUT MONENSIN FED TO SUCKLED BEEF COWS (AS-FED BASIS)

Monensin group			Control group		
cow	no. of days	ADA	cow	no. of days	ADA
1	90	2.77	2	90	2.40
6	90	2.27	3	90	2.21
7	90	2.10	5	90	2.23
10	87	2.57	8	77	2.27
12	75	2.56	9	90	2.40
13	90	2.04	11	80	2.48
15	90	2.48	14	90	2.43
16	90	2.16	17	90	2.16
20	90	2.13	18	90	2.10
21	90	2.67	19	90	2.40
22	90	2.40	24	90	2.50
23	90	2.16	26	90	2.55
25	90	2.16	27	78	2.65
28	90	2.90	29	90	2.60
mean	88.7	2.38	mean	87.5	2.38

TABLE A-2. LEAST SQUARES ANALYSES OF VARIANCE OF THE RESPONSE OF SUCKLED BEEF COWS DURING 90 DAYS POSTPARTUM TO A SUPPLEMENT (S) WITH OR WITHOUT MONENSIN AND TO A HORMONE (H) INJECTION (E_2 OR GnRH) AT 41 DAYS POSTPARTUM

Source of variation	P4[a]		CP4		Glucose		CGL		Error term
	df[b]	MS[b]	df	MS	df	MS	df	MS	
S	1	2229419	1	37566778	1	906.1	1	1454	Cow(SxH)
H[c]	1	9571643	1	14227940	1	7.2	1	363433	Cow(SxH)
SxH	1	793673	1	1158422622	1	1910.4	1	54425	Cow(SxH)
Cow(SxH)	24	17227904**	24	2067406060**	24	1426.2**	24	554119**	Residual
D[c]	1	10298816$^+$	1	1503088925**	1	2623 **	1	313703494**	Residual
DxD	1	8896164$^+$	1	1548908922**					Residual
DxDxD	1	15484562*							Residual
Residual	818	3111186	819	64080670	807	71.7	807	4523.9	

$^+$P < .10, *P < .05, **P < .01.

[a] P4 = progesterone, CP4 = cumulative P4, CGL = cumulative glucose.

[b] df = degrees of freedom, MS = mean squares.

[c] E_2 = estradiol-17β, GnRH = gonadotropin releasing hormone.

[d] D = coded day.

TABLE A-2-continued.

Source of variation	NFO[a]		CFO		OV		COV		CL		CCL		Error term
	df[b]	MS[b]	df	MS	df	MS	df	MS	df	MS	df	MS	
S	1	7.24212$^+$	1	907.5$^+$	1	1139.1$^+$	1	129420.1	1	0.04232	1	0.1895	Cow(SxH)
H	1	0.34032	1	228.1	1	10.5	1	4419.3	1	0.07678	1	0.0175	Cow(SxH)
SxH	1	0.68174	1	75.4	1	57.8	1	19767.6	1	0.09866	1	5.2802	Cow(SxH)
Cow(SxH)	24	1.91782**	24	251**	24	353.8**	24	53234.3**	24	0.25602**	24	7.0279**	Residual
D[c]	1	8.26800**	1	37.7*	1	0.313	1	239010.7**	1	2.45409**	1	67.7915**	Residual
DxD	1	1.06380*	1	1088.8**	1	37.854$^+$	1	13910.0**	1	5.4493**	1	131.374**	Residual
Residual	722	0.31049	722	7.66	722	11.876	722	777.1	722	0.06149	722	0.781372	

$^+$P <.10, *P < .05, **P < .01.

[a] NFO = no. of follicles, CFO = cumulative NFO, OV = ovarian volume, COV = cumulative OV, CL = no. of corpora lutea, CCL = cumulative CL.

[b] df = degrees of freedom, MS = mean squares.

[c] D = coded day.

TABLE A-3. LEAST SQUARES MEANS OF DEPENDENT VARIABLES MEASURED DURING 90 DAYS POSTPARTUM IN SUCKLED BEEF COWS FED A SUPPLEMENT WITH (MOS) OR WITHOUT (COS) MONENSIN AND INJECTED WITH E_2 OR GnRH AT 41 DAYS POSTPARTUM

Dependent variable	MOS	COS	E_2[a]	GnRH[a]	MOSxE_2	MOSxGnRH	COSxE_2	COSxGnRH
P4[b] (pg/ml)	1272	1375	1217	1430	1135	1409	1299	1450
CP4 (pg/ml)	14588	15921	14844	15664	13008	16168	16681	15161
Glucose (mg/dl)	71.6	69.5	70.4	70.4	70.2	73.0	71.1	67.9
CGL (mg/dl)	1106	1109	1128	1086	1101	1111	155	1062
NFO (unit)	0.666	0.469	0.546	0.589	0.615	0.717	0.478	0.461
CFO (unit)	6.413	4.209	4.759	5.863	5.543	7.283	3.975	4.444
OV (cm^3)	15.32	12.85	13.97	14.21	14.92	15.72	13.01	12.69
COV (cm^3)	199	173	184	189	192	207	172	171
NCL (unit)	0.095	0.110	0.093	0.113	0.096	0.094	0.088	0.132
CCL (unit)	0.383	0.415	0.404	0.394	0.472	0.295	0.336	0.494

[a] E2 = estradiol-17β, GnRH = gonadotropin releasing hormone.

[b] P4 = progesterone, CP4 = cumulative P4, CGL = cumulative glucose, NFO = no. of follicles, CFO = cumulative NFO, OV = ovarian volume, COV = cumulative OV, NCL = no. of corpora lutea, CCL = cumulative NCL.

TABLE A-4. TEST OF HOMOGENEITY OF REGRESSION OF THE RESPONSE OF SUCKLED BEEF COWS DURING 90 DAYS POSTPARTUM TO A SUPPLEMENT (S) WITH OR WITHOUT MONENSIN AND TO A HORMONE (H) INJECTION (E_2 OR GnRH) AT 41 DAYS POSTPARTUM

		Error term		S		Error term		H[e]		Error term		SxH	
Response	OR[a]	df[b]	MS[b]	df[c]	MS[c]	df	MS	df	MS	df	MS	df	MS
P4[d]	3	815	3119210	3	931393	815	3106672	3	4204205	809	3097003	9	4386079
CP4	2	817	63395844	2	343832126**	817	63511747	2	296485541**	813	62740409	6	245686021*
Glucose	1	806	70.75199	1	885.145**	806	71.82206	1	22.67	804	70.819699	3	327.071
CGL	1	806	451707	1	10093	806	4477.465	1	42012**	804	4247.4	3	78646.2**
NFO	2	720	0.31019	2	0.421525	720	0.307161	2	1.5095**	716	0.30699077	6	0.7291
CFO	2	720	7.1344	2	198.4416**	720	7.426647	2	93.2288**	716	6.765449	6	114.9315**
OV	2	720	11.81654	2	33.3579	720	11.8472	2	22.3163	716	11.811179	6	19.6365
COV	2	720	680.1077	2	35703.8**	720	767.8987	2	4099.05**	716	665.71879	6	14071.7**
NCL	2	720	0.061582	2	0.028312	720	0.061329	2	0.112439	716	0.0624704	6	0.06247
CCL	2	720	0.779352	2	1.50856	720	0.783376	2	0.060005	716	0.77320261	6	1.75626

*P < .05, **P < .01.

[a] OR = order of regression.

[b] Degrees of freedom (df) and mean squares (MS) associated with the error term when unpooled curves were generated for S, H, and SxH.

[c] Degrees of freedom (df) and mean squares (MS) resulted from the difference of residual sums of squares of the pooled curve minus residual sums of squares of the unpooled curves.

[d] P4 = progesterone, CP4 = cumulative P4, CGL = cumulative glucose, NFO = no. of follicles, CFO = cumulative NFO, OV = ovarian volume, COV = cumulative OV, NCL = no. of corpora lutea, CCL = cumulative NCL.

[e] E_2 = estradiol-17β, GnRH = gonadotropin releasing hormone.

TABLE A-5. LEAST SQUARES ANALYSES OF VARIANCE OF THE RESPONSE OF SUCKLED BEEF COWS DURING THE FIRST 38 DAYS POSTPARTUM TO A SUPPLEMENT (S) WITH OR WITHOUT MONENSIN

Source of variation	P4[b]		CP4		Glucose		CGL		LH		CLH		Error term
	df[a]	MS[a]	df	MS	df	MS	df	MS	df	MS	df	MS	
S	1	2098344	1	61458879	1	6.2	1	11368	1	0.000031	1	0.326	Cow(S)
Cow(S)	26	2381275**	26	103341938**	26	847**	26	78674**	26	0.263033**	26	10.623*	Residual
D[c]	1	1156874**	1	109863757**	1	56.1	1	26983345**	1	0.267732**	1	92.481*	Residual
DxD			1	18639541*									Residual
Residual	342	442058	341	4336944	338	100.8	338	1558	335	0.010848	335	0.297	

+P < .10, *P < .05, **P < .01.

[a] df = degree of freedoms, MS = mean squares.

[b] P4 = progesterone, CP4 = cumulative P4, CGL = cumulative glucose, LH = luteinizing hormone, CLH = cumulative LH.

[c] D = coded day.

TABLE A-5-continued.

Source of variation	NFO[b]		CFO		OV		COV		Error term
	df[a]	MS[a]	df	MS	df	MS	df	MS	
S	1	2.046961*	1	17.9557*	1	247.748	1	246.91	Cow(S)
Cow(S)	26	0.281397**	26	3.2016**	26	104.433**	26	104.395**	Residual
D[c]	1	6.748831**	1	4.8993**	1	93.829**	1	6.929**	Residual
DxD			1	13.9908**			1	1.513$^+$	Residual
Residual	248	0.142044	247	0.48985	248	9.2689	247	9.3003	

$^+P < .10$, $*P < .05$, $**P < .01$.

[a] df = degree of freedoms, MS = mean squares.

[b] NFO = no. of follicles, CFO = cumulative NFO, OV = ovarian volume, COV = cumulative OV.

[c] D = coded day.

TABLE A-6. LEAST SQUARES MEANS OF DEPENDENT VARIABLES MEASURED DURING THE FIRST 38 DAYS POSTPARTUM IN SUCKLED BEEF COWS FED A SUPPLEMENT WITH (MOS) OR WITHOUT (COS) MONENSIN

Dependent variable	MOS	COS
P4[a] (pg/ml)	753	885
CP4 (pg/ml)	4724	5539
Glucose (mg/dl)	72.4	72.1
CGL (mg/dl)	504	515
LH (ng/ml)	0.14	0.14
CLH (ng/ml)	0.83	0.89
NFO (unit)	0.28	0.11
CFO (unit)	0.87	0.36
OV (cm^3)	13.9	12.1
COV (cm^3)	72.2	66.1

[a] P4 = progesterone, CP4 = cumulative P4, CGL = cumulative glucose, LH = luteinizing hormone, CLH = cumulative LH, NFO = no. of follicles, CFO = cumulative NFO, OV = ovarian volume, COV = cumulative OV.

TABLE A-7. TEST OF HOMOGENEITY OF REGRESSION OF THE RESPONSE OF SUCKLED BEEF COWS DURING THE FIRST 38 DAYS POSTPARTUM TO A SUPPLEMENT (S) WITH OR WITHOUT MONENSIN

		Error term		S	
Response	OR[a]	df[b]	MS[b]	df[c]	MS[c]
P4[d]	1	341	441976	1	470121
CP4	2	339	4286589	2	12872144
Glucose	1	337	100.76	1	123.015
CGL	1	337	1541.07	1	7591.25*
LH	1	334	0.01088	1	0.00142
CLH	2	332	0.29585	2	0.04892
NFO	1	247	0.13575	1	1.69506**
CFO	2	245	0.38633	2	13.17167**
OV	1	247	8.23472	1	17.7124
COV	2	245	98.62044	2	1200.408**

*P < .05, **P < .01.

[a] Order of regression.

[b] Degress of freedom (df) and mean squares (MS) associated with the error term when unpooled curves for day trend were generated for S.

[c] Degrees of freedom (df) and mean squares (MS) obtained from the difference of residual sums of squares of the pooled day curve minus residual sums of squares of the unpooled day curves.

[d] P4 = progesterone, CP4 = cumulative P4, CGL = cumulative glucose, LH = luteinizing hormone, CLH = cumulative LH, NFO = no. of follicles, CFO = cumulative NFO, OV = ovarian volume, COV = cumulative OV.

TABLE A-8. LEAST SQUARES ANALYSES OF VARIANCE OF THE RESPONSE OF SUCKLED BEEF COWS FROM 39 TO 77 DAYS POSTPARTUM TO A SUPPLEMENT (S) WITH OR WITHOUT MONENSIN AND TO A HORMONE (H) INJECTION (E_2 OR GnRH) AT 41 DAYS POSTPARTUM

Source of variation	P4[b]		CP4		Glucose		CGL		Error term
	df[c]	MS[c]	df	MS	df	MS	df	MS	
S	1	3659	1	49374995	1	918	1	24615	Cow(SxH)
H[a]	1	16612333	1	782592684	1	2.07	1	17905	Cow(SxH)
SxH	1	8681200	1	56663539	1	893	1	81645	Cow(SxH)
Cow(SxH)	24	27568329**	24	1202995298**	24	559**	24	60263**	Residual
D[d]	1	12095124[+]	1	9483981703**	1	.82	1	23023674**	Residual
Residual	332	3423686	332	33494322	326	43.7	326	718	

[+] $P < .10$, $*P < .05$, $**P < .01$.

[a] E_2 = estradiol-17β, GnRH = gonadotropin releasing hormone.

[b] P4 = progesterone, CP4 = cumulative P4, CGL = cumulative glucose.

[c] df = degrees of freedom, MS = mean squares.

[d] D = coded day.

TABLE A-8-continued.

Source of variation	NFO[b]		CFO		OV		COV		NCL		CCL		Error term
	df[c]	MS[c]	df	MS	df	MS	df	MS	df	MS	df	MS	
S	1	3.818	1	159	1	718*	1	25396*	1	0.137	1	2.657	Cow(SxH)
H[a]	1	3.626	1	448^{+}	1	36	1	10585	1	0.029	1	0.008	Cow(SxH)
SxH	1	0.937	1	20	1	3.79	1	1808	1	0.325	1	3.679	Cow(SxH)
Cow(SxH)	24	2.769**	24	116**	24	149**	24	5755**	24	0.233**	24	2.833**	Residual
D[d]	1	8.312**	1	49**	1	218**	1	39305**	1	0.084	1	2.073*	Residual
DxD			1	25*			1	1238*	1	0.427**	1	7.074**	Residual
Residual	332	0.30254	331	3.985	332	10.72	331	202.143	331	0.049	331	0.41107	

$^{+}P < .10$, $*P < .05$, $**P < .01$.

[a] E_2 = estradiol-17β, GnRH = gonadotropin releasing hormone.

[b] NFO = no. of follicles, CFO = cumulative NFO, OV = ovarian volume, COV = cumulative OV, CL = no. of corpora lutea, CCL = cumulative CL.

[c] df = degrees of freedom, MS = mean squares.

[d] D = coded day.

TABLE A-9. LEAST SQUARES MEANS OF DEPENDENT VARIABLES MEASURED IN SUCKLED BEEF COWS FROM 39 TO 77 DAYS POSTPARTUM TO A SUPPLEMENT WITH (MOS) OR WITHOUT (COS) MONENSIN AND TO A HORMONE INJECTION (E_2 OR GnRH) AT 41 DAYS POSTPARTUM

Dependent variable	MOS	COS	E_2 [a]	GnRH [a]	MOSxE_2	MOSxGnRH	COSxE_2	COSxGnRH
P4 (pg/ml)[b]	1443	1437	1225	1655	1384	1503	1067	1807
CP4 (pg/ml)	8893	9633	7791	10736	7917	9970	7769	11503
Glucose (mg/dl)	71.4	68.2	69.7	69.8	69.7	73.1	69.7	66.7
CGL (mg/dl)	486	469	485	470	478	494	491	447
NFO (unit)	0.82	0.61	0.61	0.82	0.67	0.97	0.56	0.66
CFO (unit)	5.06	3.73	3.28	5.51	3.71	6.41	2.85	4.61
OV (cm^3)	15.6	12.8	13.9	14.5	15.2	16.0	12.6	13.0
COV (cm^3)	104	87	90	100	96	222	84	90
NCL (unit)	0.05	0.09	0.07	0.08	0.08	0.03	0.05	0.13
CCL (unit)	0.14	0.31	0.22	0.23	0.24	0.05	0.21	0.42

[a] E_2 = estradiol-17β, GnRH = gonadotropin releasing hormone.
[b] P4 = progesterone, CP4 = cumulative P4, CGL = cumulative glucose, NFO = no. of follicles, CFO = cumulative NFO, OV = ovarian volume, COV = cumulative OV, NCL = no. of corpora lutea, CCL = cumulative NCL.

TABLE A-10. TEST OF HOMOGENEITY OF REGRESSION OF THE RESPONSE OF SUCKLED BEEF COWS FROM 39 TO 77 DAYS POSTPARTUM TO A SUPPLEMENT (S) WITH OR WITHOUT MONENSIN AND TO A HORMONE (H) INJECTION (E_2 OR GnRH) AT 41 DAYS POSTPARTUM

		Error term		S		Error term		H[a]		Error term		SxH	
Response	OR[b]	df[c]	MS[c]	df[d]	MS[d]	df	MS	df	MS	df	MS	df	MS
P4[e]	1	331	3423409	1	3515404	331	3433892	1	45532	329	3391063	3	7001373
CP4	1	331	33590868	1	1537571	331	32898847	1	230596652**	329	32568677	3	135006809**
Glucose	1	325	43.7655	1	22.885	325	43.8218	1	4.583	323	43.8709	3	25.45
CGL	1	325	694.59	1	8371**	325	720.12	1	74.75	323	647.3609	3	25016**
UTSIZE	1	331	23.76	1	1.8	331	22.62	1	380.9**	329	22.74	3	128.6**
NFO	1	331	0.30342	1	0.01204	331	0.29304	1	3.4478**	329	0.292929	3	1.3579**
CFO	2	329	3.8369	2	28.3189**	329	3.8603	2	24.4675**	325	3.6998	6	116.5356**
OV	1	331	10.6879	1	21.7625	331	10.5363	1	71.9045**	329	10.4582	3	39.5677
COV	2	329	175.0123	1	4665.15**	329	198.4305	2	812.862**	325	171.9225	6	1839.09**
NCL	2	329	0.049215	2	0.3172	329	0.048533	2	0.143891	324	0.047785	6	0.120826
CCL	2	329	0.404955	2	1.4171	329	0.413181	2	0.06397	325	0.39294	6	1.39312

*$P < .05$, **$P < .01$.

[a] E_2 = estradiol-17β, GnRH = gonadotropin release hormone.

[b] Order of regression.

[c] Degrees of freedom (df) and mean squares (MS) associated with the error term when unpooled curves were generated for S, H, and SxH.

[d] Degrees of freedom (df) and mean squares (MS) resulted from the difference of residual sums of squares of the pooled curve minus residual sums of squares of the unpooled curves.

[e] P4 = progesterone, CP4 = cumulative P4, CGL = cumulative glucose, UTSIZE = uterine size, NFO = no. of follicles, CFO = cumulative NFO, OV = ovarian volume, COV = cumulative OV, NCL = no. of corpora lutea, CCL = cumulative NCL.

TABLE A-11. LEAST SQUARES POLYNOMIAL REGRESSION EQUATIONS FOR THE FIRST 77 DAYS POSTPARTUM

Dependent variable	Group	B_o	B_1	B_2	R_2
P4 (pg/ml)[a]	S[b]	459.2233	49.98576D		.21
CP4(pg/ml)	S1	423.8150	397.1046D	$24.57990D^2$	.72
	S2	-794	769.7191D	$16.79023D^2$	.72
	H1	-486.1645	756.7781D	$11.04661D^2$	.72
	H2	107.1336	407.6340D	$30.31993D^2$	.72
	S1H1	837.0117	247.6430D	$26.66097D^2$	.73
	S1H2	-43.87370	554.1367D	$22.26963D^2$	.73
	S2H1	-1757.915	1265.737D	$-4.398074D^2$	.73
	S2H2	261.4977	260.9440D	$38.36141D^2$	.73
Glucose (mg/dl)	S1	72.93430	-.0869758D		.41
	S2	74.12124	-.2899311D		.41
CGL (mg/dl)	H1	37.07626	70.48146D		.98
	H2	42.41164	68.55253D		.98
	S1H1	32.45858	68.90331D		.98
	S1H2	13.74163	70.77564D		.98
	S2H1	41.98198	72.04383D		.98
	S2H2	32.72683	66.32952D		.98
NFO (unit)	S1	-.1788432	.0508063D		.43
	S2	-.2421561	.0417115D		.43
CFO (unit)	S1	-.2117195	-.1012933D	$.0238328D^2$	.79
	S2	.5695572	-.2293407D	$.0210540D^2$	.79
	H1	.7044300	-.2112196D	$.0213521D^2$	.78
	H2	.1780113	-.1403222D	$.0241258D^2$	.78
	S1H1	.0425385	-.0959785D	$.0202900D^2$	.80
	S1H2	-.3986317	-.1141682D	$.0275789D^2$	.80
	S2H1	1.186659	-.3036189D	$.0217607D^2$	.80
	S2H2	-.0528776	-.1543357D	$.0203259D^2$	.80
OV (cm^3)	S1	12.24124	.1733622D		.53
	S2	11.15396	.0923106D		.53
COV (cm^3)	S1	-38.62687	12.13487D	$.0841132D^2$	.96
	S2	-32.75611	11.08123D	$.0450895D^2$	.96
	H1	-33.18955	11.49847D	$.0565841D^2$	.96
	H2	-36.66076	11.52679D	$.0780257D^2$	.96
	S1H1	-37.32309	11.88702D	$.0730818D^2$	.96
	S1H2	-39.45856	12.32677D	$.0966915D^2$	.96
	S2H1	-30.54934	11.29736D	$.0347538D^2$	.96
	S2H2	-34.97768	10.86818D	$.0553211D^2$	.96
NCL (unit)	S	.1195042	-.0256367D	$.0011468D^2$	.26
CCL (unit)	S	.4424170	-.0928492D	$.0040283D^2$	.31

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[a] P4 = progesterone, CP4 = cumulative P4, CGL = cumulative glucose, NFO = no. of follicles, CFO = cumulative NFO, OV = ovarian volume, COV = cumulative OV, NCL = no. of corpora lutea, CCL = cumulative NCL.

[b] S = supplement, S1 = monensin supplement, S2 = control supplement, H1 = estradiol-17β, H2 = gonadotropin releasing hormone, SH = interactions.

TABLE A-12. LEAST SQUARES POLYNOMIAL REGRESSION EQUATIONS FOR THE FIRST 77 DAYS POSTPARTUM

Dependent variable	Group	B_o	B_1	B_2	B_3	B_4	B_5
UTLO (%)[a]	S[b]	-1.355128	.4700292D	$-.409803D^2$	$.0016213D^3$	$-.0000235D^4$	
UTSIZE (mm)	S	204.9029	-45.37968D	$5.514788D^2$	$-.3265494D^3$	$.0093481D^4$	$-.0001036D^5$
	H1	186.2460	-37.29478D	$4.181012D^2$	$-.2207666D^3$	$.0054070D^4$	$-.0000487D^5$
	H2	222.7635	-53.18262D	$6.808698D^2$	$-.4295667D^3$	$.0191959D^4$	$-.0001572D^5$
UTONUS	S	.8861176	.0313950D	$-.0024377D^2$	$.0000733D^3$		
CVX (mm)	S	96.15990	-19.50699D	$2.183306D^2$	$-.1200101D^3$	$.0032184D^4$	$-.0000336D^5$

[a] UTLO = uterine location, UTSIZE = uterine size, UTONUS = uterine tonus, CVX = cervix size.

[b] S = supplement, H1 = estradiol-17 , H2 = gonadotropin releasing hormone.

TABLE A-13. LEAST SQUARES POLYNOMIAL REGRESSION EQUATIONS FOR LUTEINIZING HORMONE RESPONSE OF SUCKLED BEEF COWS TO E_2 OR GnRH INJECTION AT 41 DAYS POSTPARTUM

	Gr[b]	B_o	B_1	B_2	B_3	B_4	B_5	R_2
E_2[a]	S[c]	.1218017	.2679247Hr	$-.0843406Hr^2$	$.00931432Hr^3$	$-.0004196Hr^4$	$.0000066Hr^5$	.33
GnRH[a]	S1	-.660649	-.084431Hr	$1.998240Hr^2$	$-.4902858Hr^3$	$.0429521Hr^4$	$-.001278Hr^5$	.73
	S2	.9037235	-2.74759Hr	$3.962678Hr^2$	$-.9887769Hr^3$	$.0901402Hr^4$	$-.002808Hr^5$	.73

[a] E_2 = estradiol-17β, GnRH = gonadotropin releasing hormone.
[b] Gr = group.
[c] S = supplement, S1 = monensin supplement, S2 = control supplement.

TABLE A-14. LEAST SQUARES POLYNOMIAL REGRESSION EQUATIONS FOR THE FIRST 38 DAYS POSTPARTUM

Dependent variable	Group	B_o	B_1	B_2	R_2
P4 (pg/ml)[a]	S[b]	492.5	46.38365D		.33
CP4(pg/ml)	S	-120.0258	605.9297D	$17.5977D^2$	.81
Glucose (mg/dl)	S	72.95735	-.1025949D		.40
CGL (mg/dl)	S1	22.29302	69.95585D		.98
	S2	16.95927	72.34250D		.98
LH (ng/ml)	S	.0917622	.0071375D		.66
CLH (ng/ml)	S	.934750	.0708383D	$.0044941D^2$	.79
NFO (unit)	S1	-.4313123	.0832184D		.35
	S2	-.1322519	.0281596D		.35
CFO (unit)	S1	1.190639	-.4817938D	$.0458214D^2$	.64
	S2	.3017260	-.1308941D	$.0144567D^2$	.64
OV (cm^3)	S	11.29924	.2047389D		.57
COV (cm^3)	S1	-36.25452	10.74982D	$.2023744D^2$	.95
	S2	-32.00064	10.61717D	$.0899727D^2$	.95

[a] P4 = progesterone, CP4 = cumulative P4, CGL = cumulative glucose, LH = luteinizing hormone, CLH = cumulative LH, NFO = no. of follicles, CFO = cumulative NFO, OV = ovarian volume, COV = cumulative OV.

[b] S = supplement, S1 = monensin supplement, S2 = control supplement.

TABLE A-15. LEAST SQUARES POLYNOMIAL REGRESSION EQUATIONS FOR THE 39 TO 77 DAYS POSTPARTUM PERIOD

Dependent variable	Group	B_0	B_1	B_2	R_2
P4 (pg/ml)[b]	S[c]	1096.419	49.01599D		.38
CP4(pg/ml)	H1	-370.9784	1159.675D		.78
	H2	-438.2814	1587.726D		.78
	S1H1	-1754.627	1360.381D		.78
	S1H2	-20.40738	1419.889D		.78
	S1H1	1041.138	956.2008D		.78
	S2H2	-867.4195	1756.875D		.78
Glucose (mg/dl)	S	70.67402	-.1309781D		.52
CGL (mg/dl)	H1	-17.09926	70.55569D		.99
	H2	-14.89926	67.91539D		.99
	S1H1	-11.30131	58.61504D		.99
	S1H2	-23.65362	72.57227D		.99
	S2H1	-4.552599	69.59357D		.99
	S2H2	-24.11264	66.11612D		.99
NFO (unit)	S	.42791	.0407132D		.45
	H1	.1387477	.0673626D		.47
	H2	.7087945	.0149137D		.47
	S1H1	.2587525	.0577265D		.47
	S1H2	.7747253	.0274725D		.47
	S2H1	.0210902	.0767894D		.47
	S2H2	.6428637	.0023548D		.47
CFO (unit)	S1	.0745196	.4803910D	.0252259D^2	.83
	S2	-.0436920	.3789065D	.0174262D^2	.83
	H1	.0247101	.1499099D	.0347478D^2	.83
	H2	.0884113	.6907735D	.0088838D^2	.83
	S1H1	.0476581	.2052064D	.0349734D^2	.83
	S2H1	.2157834	.7296989D	.0168403D^2	.83
	S2H1	-.0086019	.0994434D	.0342679D^2	.83
	S2H2	-.0390160	.6518482D	.0009276D^2	.83
OV (cm^3)	S1	13.67717	.0274721D		.56
	S2	11.78293	.1429665D		.56
	H1	11.55344	.3301880D		.57
	H2	13.87560	.0906687D		.57
	S1H1	11.86726	.4694251D		.57
	S1H2	15.40593	.0888295D		.57
	S2H1	11.20996	.1939750D		.57
	S2H2	12.34528	.0930424D		.57
COV (cm^3)	S1	1.531877	12.65201D	.2080426D^2	.95
	S2	-.4960615	11.64392D	.0876104D^2	.95
	H1	2.246059	10.17843D	.2545335D^2	.95
	H2	-.6125159	13.98236D	.0482321D^2	.95
	S1H1	2.880584	10.10497D	.3486319D^2	.95
	S1H2	.817527	15.05557D	.0750050D^2	.95
	S2H1	1.229516	10.33830D	.1558874D^2	.95
	S2H2	-2.041558	12.90915D	.0214591D^2	.95

TABLE A-15-continued.

[a] E_2 = estradiol-17β, GnRH = gonadotropin releasing hormone.

[b] P4 = progesterone, CP4 = cumulative P4, CGL = cumulative glucose, NFO = no. of follicles, CFO = cumulative NFO, OV = ovarian volume, COV = cumulative OV.

[b] S = supplement, S1 = monensin supplement, S2 = control supplement, H1 = estradiol-17β, H2 = gonadotropin releasing hormone, SH = interactions.

TABLE A-16. FEED COMPOSITION OF THE CONCENTRATE MIXTURE AND HAY ON A DRY-MATTER BASIS

	Crude protein (%)	TDN[a] (%)
Corn (IFN-4-26-023) plus soybean meal (IFN-S-04-600)	16.6	85
Costal Bermudagrass hay (IFN-1-00-716)	4.6	54

[a] TDN = total digestible nutrients (NRC, 1982).

TABLE A-17 POSTPARTUM DATA

COW NUMBER=1 TREATMENT=11 BREED=3

DAY	CVX	LHD	RHD	UTL	TCN	LOL	LOH	LOW	LEFT OVARY	LST	LSL	ROL	ROH	ROW	RIGHT OVARY	RST	RSL	PROGESTERONE	GLUCOSE	LH	COW WT	CF WT	CON SCORE
1	.	.	.	.	.	.	.	.	.	.		.	.	.	.	.		437	72	0.08	449	36	6.0
2	.	.	.	.	.	.	.	.	.	.		.	.	.	.	.		366	73	0.00	.	.	.
5	.	.	.	.	.	.	.	.	.	.		.	.	.	.	.		533	64	0.00	.	.	.
8	.	.	.	.	.	.	.	.	.	.		.	.	.	.	.		750	74	0.00	.	.	.
11	55	38	40	.0	1	24	18	14	6.0	0		26	16	14	5.8	0		285	74	0.05	.	.	.
14	54	48	54	.0	1	24	20	14	6.7	0		26	20	16	8.3	0		549	72	0.05	440	.	.
17	42	34	36	.0	1	24	20	16	7.7	0		22	16	14	4.9	0		350	51	0.05	.	.	.
20	38	36	40	.0	1	28	22	16	9.9	0		24	18	16	6.9	0		422	47	0.00	.	.	.
23	30	32	32	.5	1	24	20	16	7.7	0		28	22	20	12.3	1	MGC	666	62	0.05	.	.	.
26	36	34	36	.5	1	24	18	16	6.9	0		22	20	16	7.0	1	LM	1122	73	0.05	440	.	.
29	30	32	32	1	1	24	20	16	7.7	0		24	20	18	8.6	1	LGC	67	63	0.05	.	.	.
32	26	30	28	1	1	30	24	20	14.4	0		32	22	18	12.7	0		405	57	0.10	.	.	.
35	26	24	26	1	1	22	16	14	4.9	0		26	20	16	8.3	1	MGC	450	63	0.05	.	.	.
38	26	26	28	1	1	24	22	18	9.5	0		26	22	20	11.4	1	TM	1005	73	0.00	434	.	5.0
41	.	.	.	.	.	.	.	.	.	.		.	.	.	.	.		405	.	.	.	.	.
44	38	44	46	.5	1	22	18	14	5.5	0		24	20	16	7.7	0		694	62	.	.	.	.
47	30	34	32	.5	1	22	18	14	5.5	0		26	22	18	10.3	0		794	80	.	.	.	.
50	28	28	30	1	2	22	18	14	5.5	0		26	20	18	9.4	0		666	77	.	436	.	.
53	26	28	28	.5	1	24	20	16	7.7	0		24	20	18	8.6	1	LGC	277	73	.	.	.	.
56	32	36	34	.5	1	24	20	16	7.7	0		24	18	14	6.0	0		505	64	.	.	.	.
59	26	26	28	1	1	24	20	18	8.6	0		26	22	20	11.4	1	LM	955	66	.	.	.	.
62	26	26	28	1	1	24	20	18	8.6	0		24	18	16	6.9	0		438	57	.	452	.	.
65	26	24	26	1	1	24	22	18	9.5	1	TM	24	20	18	8.6	0		1318	69	.	.	.	.
68	28	28	30	1	1	26	22	20	11.4	1	TM	26	20	18	9.4	0		61	72	.	.	.	.
71	26	28	26	1	1	24	20	18	8.6	0		26	20	18	9.4	1	MGC	188	73	.	.	.	.
74	24	26	26	1	1	22	20	18	7.9	0		26	20	18	9.4	1	TM	300	64	.	447	.	.
77	28	28	28	1	1	22	18	16	6.3	0		24	20	18	8.6	0		111	56	.	.	.	.
80	28	28	28	1	1	24	20	18	8.6	1	TM	28	24	22	14.8	1	LM	272	67	.	.	.	.
83	28	28	30	1	1	22	20	14	6.2	1	TM	24	22	18	9.5	1	LM	544	59	.	.	.	.
86	28	28	30	.5	1	26	22	18	10.3	1	LM	24	20	18	8.6	1	TM	311	69	.	461	.	.
89	28	28	28	1	1	24	22	18	9.5	1	LM	24	20	18	8.6	1	LL	146	69	.	463	104	4.7

TABLE A-17 CONTINUED POSTPARTUM DATA

COW NUMBER=2 TREATMENT=22 BREED=4

DAY	CVX	LHC	RHC	UTL	TON	LOL	LOH	LOW	LEFT OVARY	LST	LSL	ROL	ROH	ROW	RIGHT OVARY	RST	RSL	PROGESTERONE	GLUCOSE	LH	COW WT	CF WT	CON SCORE
1	.	.	.	.	.	.	.	.	.	.		.	.	.	.	.		623	74	0.80	.	45	6.7
5	.	.	.	.	.	.	.	.	.	.		.	.	.	.	.		0	.	.	.	.	.
9	52	60	54	.0	1	20	16	14	4.5	0		22	16	14	4.9	0		1342	91	0.43	329	.	.
11	48	56	52	.0	1	22	16	12	4.2	0		20	16	14	4.5	0		1116	73	0.58	.	.	.
14	34	56	52	.0	1	24	16	12	4.6	0		22	18	14	5.5	0		1090	75	0.55	.	.	.
17	32	38	34	.0	1	24	16	12	4.6	0		22	16	14	4.9	0		1068	82	0.50	.	.	.
20	38	40	36	.0	1	22	18	14	5.5	1	MGC	22	16	14	4.9	0		1031	70	0.55	354	.	.
23	30	36	34	.5	1	24	18	14	6.0	0		22	16	14	4.9	0		746	62	0.50	.	.	.
26	32	34	30	.5	1	22	14	14	4.3	0		22	16	14	4.9	0		995	45	0.59	.	.	.
29	32	36	34	.5	1	26	18	14	6.6	0		24	18	16	6.9	0		1514	58	0.38	.	.	.
32	30	34	30	.5	1	24	18	14	6.0	0		24	20	16	7.7	0		494	73	0.40	350	.	.
35	26	28	26	1	1	22	16	12	4.2	0		24	18	16	6.9	0		810	63	0.35	.	.	.
38	26	28	26	.5	1	22	18	16	6.3	0		22	18	14	5.5	0		1079	64	0.48	.	.	.
41	28	28	26	1	1	24	20	16	7.7	0		22	20	16	7.0	1	MGC	768	62	0.53	.	.	4.0
44	26	28	26	1	1	22	16	14	4.9	1	LM	22	18	16	6.3	0		950	65	.	363	.	.
47	28	28	26	1	1	24	20	16	7.7	1	TM	20	16	14	4.5	0		1600	69	.	.	.	.
50	28	28	26	1	1	24	20	16	7.7	0		24	22	18	9.5	1	TM	2679	65	.	.	.	.
53	28	30	28	1	1	24	20	16	7.7	1	LGC	24	20	16	7.7	0		402	61	.	.	.	.
56	26	26	26	1	1	24	22	18	9.5	1	LGC	24	20	16	7.7	1	TM	268	67	.	375	.	.
59	26	28	26	1	1	24	20	18	8.6	1	TM	22	18	14	5.5	1	LM	402	55	.	.	.	.
62	26	26	26	1	1	24	20	16	7.7	1	TL	22	18	16	6.3	1	LM	135	57	.	.	.	.
65	28	30	28	1	1	22	16	14	4.9	1	LM	24	20	16	7.7	1	TM	59	58	.	.	.	.
68	26	26	26	1	1	24	16	14	5.4	1	MGC	24	20	16	7.7	1	TM	49	64	.	361	.	.
71	26	28	26	1	1	24	18	14	6.0	1	LL	22	18	14	5.5	0		236	64	.	.	.	.
74	26	26	26	1	1	24	20	14	6.7	1	MGC	24	18	14	6.0	1	TM	273	58	.	.	.	.
77	24	26	26	1	1	24	18	16	6.9	1	LM	24	18	14	6.0	0		300	67	.	.	.	.
80	26	28	28	1	1	22	20	16	7.0	1	MGC	22	20	16	7.0	0		306	66	.	375	.	.
83	26	28	26	1	1	24	20	16	7.7	1	MCC	22	20	16	7.0	0		198	60	.	.	.	.
86	26	26	26	1	1	22	16	14	4.9	1	TM	24	16	14	5.4	0		311	62	.	.	.	.
89	26	26	28	1	1	22	18	14	5.5	1	TM	22	16	14	4.9	0		236	62	.	390	113	5.0

TABLE A-17 CONTINUED POSTPARTUM DATA

COW NUMBER=3 TREATMENT=21 BREED=1

DAY	CVX	LHO	RHO	UTL	TON	LOL	LOH	LOW	LEFT OVARY	LST	LSL	ROL	ROH	ROW	RIGHT OVARY	RST	RSL	PROGESTERONE	GLUCOSE	LH	COW WT	CF WT	CON SCORE
1	.	.	.	.	.	.	.	.	.	.		.	.	.	.	.		1652	71	0.00	352	36	7.3
4	.	.	.	.	.	.	.	.	.	.		.	.	.	.	.		1105	64	0.00	.	.	.
7	.	.	.	.	.	.	.	.	.	.		.	.	.	.	.		115	84	0.10	.	.	.
10	50	46	48	.0	1	18	14	12	3.0	0		18	14	12	3.0	0		400	59	0.00	359	.	.
13	34	34	36	.0	1	22	20	16	7.0	0		24	18	16	6.9	0		315	64	0.00	.	.	.
16	26	24	28	.5	1	22	20	16	7.0	0		22	18	14	5.5	0		300	58	0.00	.	.	.
19	32	34	32	1	1	18	20	16	5.8	1	LGC	22	20	16	7.0	0		552	61	0.13	.	.	.
22	30	30	34	.0	1	24	18	14	6.0	0		28	20	16	9.0	0		563	69	0.05	350	.	.
25	32	34	38	.5	1	22	16	12	4.2	0		22	20	16	7.0	0		2831	68	0.05	.	.	.
28	24	26	24	1	1	24	18	14	6.0	0		28	22	20	12.3	0		363	68	0.05	.	.	.
31	28	30	34	1	1	22	12	10	2.6	0		20	16	14	4.5	0		2299	88	0.00	.	.	.
34	26	28	30	1	1	24	18	14	6.0	0		24	20	16	7.7	0		431	68	0.53	361	.	.
37	26	26	28	1	1	20	16	14	4.5	0		24	18	14	6.0	0		999	63	0.13	.	.	.
40	26	28	30	1	1	22	14	12	3.7	0		22	16	14	4.9	0		1363	61	0.00	.	.	5.0
43	26	26	28	.5	1	20	14	12	3.4	0		22	16	14	4.9	0		294	58	.	.	.	.
46	26	26	28	1	2	22	16	14	4.9	0		22	18	14	5.5	1	LM	1352	68	.	329	.	.
49	26	28	26	1	1	22	18	14	5.5	0		24	20	16	7.7	0		647	65	.	.	.	.
52	28	30	28	1	1	20	16	14	4.5	1	LM	24	20	16	7.7	1	TM	573	58	.	.	.	.
55	26	26	28	1	1	22	16	14	4.9	0		24	20	16	7.7	0		226	72	.	.	.	.
58	28	28	26	1	1	24	20	16	7.7	0		22	18	14	5.5	0		126	72	.	356	.	.
61	26	26	28	.5	1	24	16	14	5.4	0		22	16	14	4.9	0		205	68	.	.	.	.
64	24	26	26	1	1	20	16	12	3.8	0		24	20	16	7.7	1	RGC	342	66	.	.	.	.
67	28	28	30	1	1	24	20	16	7.7	0		24	20	18	8.6	0		1989	68	.	.	.	.
70	28	30	28	1	1	24	16	14	5.4	0		22	18	16	6.3	1	TM	363	61	.	379	.	.
73	26	26	26	1	2	24	18	16	6.9	1	MGC	22	18	16	6.3	1	TM	378	81	.	.	.	.
76	24	26	26	1	2	22	18	14	5.5	0		24	20	16	7.7	1	LM	957	64	.	.	.	.
79	26	24	26	1	1	22	20	16	7.0	1	MGC	20	18	16	5.8	1	TM	3078	62	.	.	.	.
82	28	28	30	.5	2	24	22	20	10.6	1	RGC	24	20	18	8.6	2	LGC	5626	59	.	384	.	.
85	28	26	28	1	2	26	20	16	8.3	1	RGC	26	20	16	8.3	2	LGC	6047	60	.	.	.	.
88	28	28	28	1	2	24	18	14	6.0	0		22	22	18	8.7	2	MGC	263	61	.	.	.	.
91	26	26	26	1	1	20	16	12	3.8	1	LR	22	18	14	5.5	0		456	64	.	361	100	6.3

TABLE A-17 CONTINUED POSTPARTUM DATA

COW NUMBER=5 TREATMENT=22 BREED=2

DAY	CVX	LHO	RHO	UTL	TON	LOL	LOH	LOW	LEFT OVARY	LST	LSL	ROL	ROH	ROW	RIGHT OVARY	RST	RSL	PROGESTERONE	GLUCOSE	LH	COW WT	CF WT	CON SCORE
0	.	.	.	.	.	.	.	.	.	.		.	.	.	.	.		360	69	0.00	352	32	5.7
3	.	.	.	.	.	.	.	.	.	.		.	.	.	.	.		256	76	0.00	.	.	.
6	.	.	.	.	.	.	.	.	.	.		.	.	.	.	.		259	64	0.10	.	.	.
9	50	35	45	.0	1	20	12	10	2.4	0		26	20	16	8.3	0		231	75	0.00	.	.	.
12	42	36	40	.0	1	22	18	14	5.5	0		26	22	20	11.4	0		318	58	0.00	363	.	.
15	38	34	38	.0	1	22	16	12	4.2	0		22	18	14	5.5	0		241	66	0.30	.	.	.
18	36	32	34	.5	1	24	20	16	7.7	0		24	18	16	6.9	0		408	74	0.00	.	.	.
21	30	26	28	.5	1	22	16	14	4.9	0		26	20	16	8.3	0		626	63	0.00	.	.	.
24	26	26	28	.5	1	24	20	16	7.7	0		22	20	18	7.9	0		233	65	0.00	363	.	.
27	24	24	28	.5	1	22	18	16	6.3	0		26	20	16	8.3	0		412	58	0.00	.	.	.
30	28	28	30	.5	1	22	16	14	4.9	0		26	22	18	10.3	0		686	68	0.00	.	.	.
33	26	26	28	.5	1	22	20	18	7.9	1	TM	24	22	20	10.6	0		494	73	0.10	.	.	.
36	26	26	28	.5	1	22	20	16	7.0	0		26	20	16	8.3	0		330	78	0.00	352	.	.
39	28	28	32	.5	1	22	18	16	6.3	0		26	22	18	10.3	0		1387	79	0.00	.	.	.
42	26	28	30	1	1	20	16	14	4.5	0		22	18	14	5.5	0		309	.	.	.	.	4.7
45	24	24	26	1	1	22	16	14	4.9	0		22	16	12	4.2	0		0	.	.	.	.	.
48	24	22	24	1	2	22	20	18	7.9	1	LGC	24	20	16	7.7	1	MGC	1672	61	.	368	.	.
51	24	24	24	1	1	24	20	18	8.6	1	MGC	22	16	14	4.9	0		3723	52	.	.	.	.
54	30	32	34	1	1	24	20	18	8.6	0		26	20	16	8.3	0		280	60	.	.	.	.
57	24	22	26	.5	1	24	20	16	7.7	1	LGC	26	22	16	9.2	0		1043	76	.	.	.	.
60	24	26	26	.5	1	22	16	14	4.9	0		22	18	16	6.3	0		139	73	.	375	.	.
63	26	26	26	.5	1	22	20	16	7.0	0		26	20	18	9.4	1	LM	433	66	.	.	.	.
66	26	26	28	1	1	24	18	16	6.9	1	TM	26	22	18	10.3	1	MGC	233	73	.	.	.	.
69	30	32	34	1	1	20	16	14	4.5	0		24	20	16	7.7	1	MGC	288	72	.	.	.	.
72	24	26	26	1	1	22	20	18	7.9	1	TM	26	24	18	11.2	0		712	69	.	381	.	.
75	26	26	28	.5	1	22	18	14	5.5	0		24	20	18	8.6	0		985	67	.	.	.	.
78	30	28	30	.5	1	20	16	14	4.5	1	LGC	22	20	16	7.0	0		362	68	.	.	.	.
81	26	24	26	1	1	20	16	14	4.5	1	LM	24	20	18	8.6	2	LGC	1303	70	.	.	.	.
84	26	24	26	1	2	20	14	12	3.4	0		26	24	22	13.7	2	LGC	6235	64	.	377	.	.
87	26	28	26	.5	2	22	18	14	5.5	0		32	26	22	18.3	2	LGC	8041	66	.	.	.	.
90	26	26	26	.5	2	20	16	14	4.5	1	MGC	32	26	24	20.0	2	LGC	10080	63	.	386	104	5.3

TABLE A-17 CONTINUED POSTPARTUM DATA

COW NUMBER=6 TREATMENT=12 BREED=4

DAY	CVX	LHO	RHO	UTL	TON	LOL	LCH	LOW	LEFT OVARY	LST	LSL	ROL	ROH	ROW	RIGHT OVARY	RST	RSL	PROGESTERONE	GLUCOSE	LH	COW WT	CF WT	CON SCORE
0	.	.	.	.	.	.	.	.	.	.		.	.	.	.	.		639	71	0.15	352	41	5.0
2	.	.	.	.	.	.	.	.	.	.		.	.	.	.	.		805	70	0.08	.	.	.
5	.	.	.	.	.	.	.	.	.	.		.	.	.	.	.		241	58	0.00	.	.	.
8	.	.	.	.	.	.	.	.	.	.		.	.	.	.	.		1278	70	0.05	.	.	.
11	40	42	40	.0	1	22	18	14	5.5	0		18	14	12	3.0	0		768	64	0.00	.	.	.
14	30	26	34	1	1	22	16	14	4.9	0		24	20	16	7.7	0		633	70	0.05	329	.	.
17	32	34	34	.0	1	24	18	13	5.6	0		22	16	14	4.9	0		907	63	0.10	.	.	.
20	32	36	32	.0	1	22	16	14	4.9	0		22	14	12	3.7	0		682	60	0.10	.	.	.
23	24	24	26	.5	1	24	16	12	4.6	0		20	18	14	5.0	1	RGC	338	66	0.00	.	.	.
26	38	40	42	.0	1	20	16	14	4.5	0		22	16	14	4.9	0		182	75	0.05	334	.	.
29	30	32	36	.5	1	22	16	12	4.2	0		24	20	16	7.7	0		365	72	0.00	.	.	.
32	26	28	26	.5	1	24	22	18	9.5	0		24	20	16	7.7	1	TM	461	66	0.00	.	.	.
35	32	34	32	.5	1	22	18	14	5.5	1	LM	24	20	16	7.7	0		692	67	0.13	.	.	.
38	26	24	26	.5	1	22	16	12	4.2	1	MGC	20	18	14	5.0	0		1637	69	0.05	343	.	3.0
41	26	28	26	1	1	24	18	16	6.9	0		24	18	14	6.0	0		434	66	.	.	.	.
44	24	26	24	.5	1	24	20	16	7.7	0		22	18	14	5.5	1	RGC	537	66	.	.	.	.
47	24	26	24	.5	1	24	20	16	7.7	0		22	18	14	5.5	0		268	63	.	.	.	.
50	26	28	26	1	1	22	18	14	5.5	0		24	18	16	6.9	1	LM	94	64	.	340	.	.
53	26	28	26	.5	1	20	18	14	5.0	1	MGC	22	18	14	5.5	1	MGC	214	71	.	.	.	.
56	26	28	26	.5	1	24	18	14	6.0	0		24	20	18	8.6	1	RGC	91	63	.	.	.	.
59	26	28	28	1	1	20	16	12	3.8	0		22	18	14	5.5	1	LR	150	63	.	.	.	.
62	26	28	26	1	1	20	18	16	5.8	1	LL	20	16	14	4.5	0		386	66	.	365	.	.
65	26	26	26	1	1	24	18	14	6.0	0		22	18	16	6.3	1	RGC	295	61	.	.	.	.
68	26	26	28	1	1	24	20	16	7.7	1	MGC	22	18	14	5.5	1	LR	343	62	.	.	.	.
71	28	28	28	1	1	22	20	16	7.0	1	TM	24	20	18	8.6	1	LM	273	62	.	.	.	.
74	26	28	26	1	1	24	16	14	5.4	1	TM	24	16	14	5.4	1	LR	129	62	.	359	.	.
77	24	26	24	1	1	20	16	14	4.5	1	TM	22	16	14	4.9	1	MGC	241	56	.	.	.	.
80	26	28	26	1	1	24	20	18	8.6	1	TM	24	20	16	7.7	1	LM	155	66	.	.	.	.
83	28	28	26	1	1	24	18	14	6.0	1	TM	24	20	18	8.6	1	MGC	322	58	.	.	.	.
86	24	26	26	1	1	22	16	14	4.9	1	TM	22	20	16	7.0	1	LL	349	65	.	370	.	.
89	26	26	26	1	1	22	16	14	4.9	0		22	18	16	6.3	1	LM	241	56	.	368	79	5.0

TABLE A-17 CONTINUED POSTPARTUM DATA

COW NUMBER=7 TREATMENT=11 BREED=4

DAY	CVX	LHO	RHO	UTL	TON	LOL	LOH	LOW	LEFT OVARY	LST	LSL	ROL	ROH	ROW	RIGHT OVARY	RST	RSL	PROGESTERONE	GLUCOSE	LH	COW WT	CF WT	CON SCORE
0	.	.	.	.	.	.	.	.	.	.		.	.	.	.	.		385	79	0.00	304	23	5.0
2	.	.	.	.	.	.	.	.	.	.		.	.	.	.	.		184	66	0.00	.	.	.
5	.	.	.	.	.	.	.	.	.	.		.	.	.	.	.		356	60	0.00	.	.	.
9	46	48	44	.0	1	24	16	14	5.4	0		24	16	12	4.6	0		1241	64	0.00	.	.	.
12	38	36	32	.0	1	20	14	12	3.4	0		20	16	12	3.8	0		1563	81	0.00	304	.	.
15	32	30	32	.5	1	24	20	14	6.7	0		26	20	16	8.3	0		172	56	0.00	.	.	.
18	30	28	30	.5	1	22	20	16	7.0	0		24	18	16	6.9	0		143	57	0.00	.	.	.
21	24	22	24	.5	1	22	18	14	5.5	0		20	16	14	4.5	0		112	57	0.00	.	.	.
24	28	26	28	.5	1	22	18	14	5.5	0		22	18	16	6.3	0		172	56	0.00	325	.	.
27	26	26	24	1	1	20	18	14	5.0	0		24	20	16	7.7	0		116	51	0.00	.	.	.
30	24	24	24	1	1	24	20	16	7.7	0		22	18	12	4.8	0		173	63	0.00	.	.	.
33	26	24	26	.5	1	20	16	12	3.8	0		24	20	16	7.7	1	TM	948	64	0.00	.	.	.
36	26	28	28	1	1	22	16	12	4.2	0		22	18	14	5.5	0		2212	160	0.00	327	.	.
39	24	26	28	1	1	18	16	12	3.5	0		18	16	14	4.0	0		500	62	0.00	.	.	.
42	24	26	26	1	1	22	16	12	4.2	0		24	18	14	6.0	0		799	64	.	.	.	4.7
45	24	26	28	1	1	18	14	10	2.5	0		22	16	12	4.2	0		344	63	.	.	.	.
48	28	30	34	.5	1	18	14	12	3.0	0		16	14	12	2.7	0		264	57	.	.	.	.
51	26	26	28	1	1	22	16	14	4.9	0		20	16	12	3.8	0		1252	56	.	316	.	.
54	24	24	26	1	1	24	16	14	5.4	0		22	18	14	5.5	0		178	56	.	.	.	.
57	26	28	28	1	1	22	18	16	6.3	1	TM	20	18	14	5.0	0		12	60	.	.	.	.
60	26	28	28	1	1	22	18	14	5.5	0		24	20	16	7.7	0		43	59	.	.	.	.
63	24	28	26	1	1	22	16	14	4.9	0		24	20	18	8.6	1	TM	57	62	.	331	.	.
66	24	26	26	1	1	22	16	14	4.9	0		20	16	14	4.5	0		109	64	.	.	.	.
69	24	26	26	1	1	22	16	14	4.9	0		24	20	16	7.7	0		189	61	.	.	.	.
72	24	26	26	1	1	22	18	16	6.3	0		26	22	18	10.3	1	LM	63	66	.	.	.	.
75	24	26	24	1	1	20	16	14	4.5	0		22	20	16	7.0	1	TM	15	63	.	354	.	.
78	24	24	26	1	1	20	16	14	4.5	0		22	18	16	6.3	0		258	65	.	.	.	.
81	26	26	26	1	1	22	14	12	3.7	1	TM	22	18	16	6.3	0		69	62	.	.	.	.
84	24	26	24	1	1	20	16	14	4.5	0		22	20	16	7.0	1	TM	74	65	.	.	.	.
87	24	26	26	1	1	20	16	14	4.5	1	MGC	24	20	16	7.7	1	TM	126	57	.	350	.	.
90	26	26	26	1	1	20	16	14	4.5	1	TL	22	20	16	7.0	1	TM	64	60	.	331	70	5.0

TABLE A-17 CONTINUED POSTPARTUM DATA

COW NUMBER=8 TREATMENT=22 BREED=1

D A Y	C V X	L H O	R H G	U T L	T O N	L O L	L O H	L O W	LEFT OVARY	L S T	L S L	R O L	R O H	R O W	RIGHT OVARY	R S T	R S L	PRO GESTE RONE	GLU CO SE	LH	COW WT	CF WT	CON SCO RE
0	.	.	.	.	.	.	.	.	.	.		.	.	.	.	.		749	62	0.05	345	30	6.0
1	.	.	.	.	.	.	.	.	.	.		.	.	.	.	.		538	59	0.00	.	.	.
4	.	.	.	.	.	.	.	.	.	.		.	.	.	.	.		316	58	0.05	.	.	.
7	.	.	.	.	.	.	.	.	.	.		.	.	.	.	.		255	60	0.00	.	.	.
10	38	40	44	.0	1	22	16	14	4.9	0		22	18	14	5.5	0		189	57	0.05	.	.	.
13	32	34	32	.5	1	26	20	16	8.3	0		24	18	14	6.0	0		200	61	0.13	356	.	.
16	32	32	34	.5	1	24	16	14	5.4	0		22	16	14	4.9	0		205	62	0.05	.	.	.
19	32	34	32	.5	1	26	18	14	6.6	0		24	16	14	5.4	0		261	59	0.10	.	.	.
22	32	32	34	1	1	22	16	14	4.9	0		20	14	12	3.4	0		899	75	0.30	.	.	.
25	28	32	30	.5	1	24	18	14	6.0	0		22	16	14	4.9	0		124	58	0.10	352	.	.
28	30	32	34	1	1	24	18	16	6.9	0		24	18	14	6.0	0		282	60	0.30	.	.	.
31	28	32	30	1	1	24	20	16	7.7	0		22	20	18	7.9	1	LM	411	58	0.25	.	.	.
34	30	32	32	1	1	22	16	14	4.9	0		22	14	12	3.7	0		405	61	0.28	.	.	.
37	32	34	32	1	1	24	18	16	6.9	1	TM	26	20	16	8.3	1	MGC	2049	57	0.30	352	.	6.3
40	32	36	34	.5	1	26	18	14	6.6	0		20	16	14	4.5	0		294	59	.	.	.	.
43	26	26	28	1	1	26	20	18	9.4	1	LGC	24	20	18	8.6	0		1505	59	.	.	.	.
46	26	28	26	.5	1	26	22	20	11.4	1	RGC	24	20	18	8.6	0		3438	58	.	.	.	.
49	30	30	32	.5	1	26	22	20	11.4	1	LM	24	20	16	7.7	0		7515	66	.	345	.	.
52	28	30	28	1	1	24	20	18	8.6	1	LGC	22	18	14	5.5	0		6604	62	.	.	.	.
55	30	36	32	.5	1	24	22	18	9.5	1	LGC	20	16	14	4.5	0		9099	62	.	.	.	.
58	28	30	28	.5	1	26	20	16	8.3	1	MGC	20	14	12	3.4	0		250	54	.	.	.	.
61	30	30	32	1	1	20	16	12	3.8	0		24	18	16	6.9	0		188	61	.	336	.	.
64	26	26	28	1	2	26	20	16	8.3	2	MGC	22	16	14	4.9	0		2249	62	.	.	.	.
67	24	26	26	1	1	26	22	18	10.3	2	MGC	20	16	14	4.5	0		4110	58	.	.	.	.
70	26	26	24	1	2	26	20	16	8.3	2	MGC	22	18	14	5.5	1	LM	11103	64	.	.	.	.
73	28	30	28	1	2	26	18	16	7.5	2	MGC	24	16	14	5.4	0		10593	60	.	375	.	.
76	26	28	28	1	2	24	20	18	8.6	2	MGC	22	20	16	7.0	1	MGC	7027	57	.	386	79	6.0

TABLE A-17 CONTINUED POSTPARTUM DATA

COW NUMBER=9 TREATMENT=21 BREED=1

D A Y	C V X	L H O	R H O	U T L	T O N	L O L	L O H	L O W	LEFT OVARY	L S T	L S L	R O L	R O H	R O W	RIGHT OVARY	R S T	R S L	PRO GESTE RONE	GLU CO SE	LH	COW WT	CF WT	CON SCO RF
0	.	.	.	.	.	.	.	.	.	.		.	.	.	.	.		306	98	0.43	375	41	7.0
2	.	.	.	.	.	.	.	.	.	.		.	.	.	.	.		838	95	0.25	.	.	.
5	.	.	.	.	.	.	.	.	.	.		.	.	.	.	.		1090	109	0.23	.	.	.
8	.	.	.	.	.	.	.	.	.	.		.	.	.	.	.		2701	124	0.53	.	.	.
11	34	34	36	.0	1	24	16	14	5.4	0		20	16	14	4.5	0		1294	92	0.33	.	.	.
14	38	40	44	.5	1	22	16	14	4.9	0		22	16	12	4.2	0		1197	110	0.38	347	.	.
17	28	28	30	.5	1	22	16	14	4.9	1	TM	20	16	14	4.5	0		1084	73	0.38	.	.	.
20	28	30	32	.5	1	22	16	12	4.2	0		20	14	12	3.4	0		972	64	0.40	.	.	.
23	28	26	30	1	1	20	18	14	5.0	0		22	18	16	6.3	0		1659	92	0.18	.	.	.
26	26	26	28	1	1	22	18	14	5.5	0		22	16	12	4.2	0		1165	89	0.30	347	.	.
29	26	26	26	1	1	20	14	12	3.4	0		20	16	14	4.5	0		1004	90	0.40	.	.	.
32	26	28	30	.5	1	22	16	14	4.9	0		22	20	18	7.9	1	TR	1546	98	0.35	.	.	.
35	28	28	30	.5	1	20	14	12	3.4	0		22	18	14	5.5	1	MGC	1396	86	0.38	.	.	.
38	26	26	28	1	1	20	16	14	4.5	0		24	22	18	9.5	0		1294	77	0.20	377	.	5.3
41	28	28	26	1	1	20	14	12	3.4	0		22	16	14	4.9	0		311	91	.	.	.	.
44	26	28	26	1	2	20	14	12	3.4	0		20	16	12	3.8	1	TM	2185	90	.	.	.	.
47	30	32	32	1	2	20	16	12	3.8	1	TM	22	18	14	5.5	0		408	75	.	.	.	.
50	28	28	30	1	1	20	16	14	4.5	0		20	16	14	4.5	1	TM	2126	84	.	354	.	.
53	28	30	30	1	1	20	14	12	3.4	0		22	18	14	5.5	1	TM	402	79	.	.	.	.
56	28	30	28	1	1	20	16	12	3.8	1	TR	22	16	14	4.9	1	LM	268	83	.	.	.	.
59	28	28	28	1	1	20	14	12	3.4	1	TM	22	18	14	5.5	0		1605	75	.	.	.	.
62	26	26	26	1	1	16	14	12	2.7	0		20	18	14	5.0	1	TM	204	76	.	370	.	.
65	24	26	26	1	1	20	16	12	3.8	1	LR	22	18	14	5.5	1	LM	467	71	.	.	.	.
68	24	24	24	1	1	22	16	14	4.9	0		20	14	12	3.4	0		1014	89	.	.	.	.
71	24	24	24	1	1	20	14	12	3.4	1	TM	22	16	14	4.9	0		402	68	.	.	.	.
74	26	28	28	1	1	20	14	12	3.4	0		18	14	12	3.0	0		204	71	.	381	.	.
77	26	26	26	1	1	20	16	14	4.5	0		22	16	14	4.9	1	TM	187	73	.	.	.	.
80	24	26	26	1	1	22	18	14	5.5	1	TM	20	16	14	4.5	1	TM	2330	75	.	.	.	.
83	24	26	26	1	1	22	18	16	6.3	1	TM	20	14	12	3.4	0		5278	80	.	.	.	.
86	26	26	26	1	1	20	18	14	5.0	1	MGC	18	14	12	3.0	0		1804	65	.	386	.	.
89	26	28	30	1	2	22	16	14	4.9	1	LGC	20	16	14	4.5	0		434	70	.	390	104	6.3

TABLE A-17 CONTINUED POSTPARTUM DATA

COW NUMBER=10 TREATMENT=12 BREED=3

DAY	CVX	LHO	RHO	UTL	TON	LOL	LOH	LOW	LEFT OVARY	LST	LSL	ROL	ROH	ROW	RIGHT OVARY	RST	RSL	PROGESTERONE	GLUCOSE	LH	COW WT	CF WT	CON SCORE
1	.	.	.	.	.	.	.	.	.	.		.	.	.	.	.		626	72	0.00	395	32	5.7
5	.	.	.	.	.	.	.	.	.	.		.	.	.	.	.		252	59	0.00	.	.	.
8	.	.	.	.	.	.	.	.	.	.		.	.	.	.	.		373	53	0.00	.	.	.
12	54	40	48	.0	1	24	16	14	5.4	0		32	22	18	12.7	0		252	56	0.18	413	.	.
15	40	32	34	.0	1	24	20	16	7.7	0		26	22	18	10.3	0		442	56	0.00	.	.	.
18	36	36	34	.5	1	24	20	16	7.7	0		26	20	16	8.3	0		92	59	0.00	.	.	.
21	32	30	28	.5	1	24	20	18	8.6	0		24	20	18	8.6	0		408	58	0.00	.	.	.
24	28	28	26	.5	1	30	24	20	14.4	0		32	24	20	15.4	0		310	62	0.20	413	.	.
27	28	28	26	1	1	26	22	18	10.3	0		34	24	20	16.3	0		270	58	0.15	.	.	.
30	28	32	30	.5	1	28	24	20	13.4	0		32	26	20	16.6	0		149	61	0.00	.	.	.
33	24	24	26	.0	1	24	22	18	9.5	1	TM	28	24	20	13.4	0		150	66	0.00	.	.	.
36	26	26	28	.5	1	20	18	16	5.8	0		24	20	16	7.7	0		362	67	0.10	402	.	.
39	28	30	28	.5	1	24	22	20	10.6	0		26	20	18	9.4	0		363	67	0.35	.	.	4.0
42	26	26	28	.5	1	24	22	18	9.5	1	LM	26	20	18	9.4	0		201	.	.	.	.	.
45	26	28	26	.5	1	24	22	18	9.5	1	LGC	28	22	20	12.3	0		224	61	.	.	.	.
48	26	26	28	.5	1	24	20	18	8.6	0		26	24	23	14.4	1	MGC	367	60	.	409	.	.
51	26	28	28	.5	1	24	18	16	6.9	1	LM	28	22	18	11.1	0		810	62	.	.	.	.
54	26	26	28	.5	1	24	22	18	9.5	1	MGC	26	24	20	12.5	0		816	58	.	.	.	.
57	32	34	36	.5	1	24	22	20	10.6	1	LM	28	22	20	12.3	0		350	63	.	.	.	.
60	28	26	28	1	1	26	20	18	9.4	1	TM	26	22	20	11.4	0		954	63	.	400	.	.
63	26	24	26	1	1	26	24	24	15.0	1	LM	26	20	20	10.4	1	TM	6069	70	.	.	.	.
66	28	26	28	.5	1	34	30	28	28.6	1	LL	26	22	20	11.4	0		8298	64	.	.	.	.
69	28	30	32	.5	1	34	26	22	19.4	1	MGC	26	22	20	11.4	0		9758	64	.	.	.	.
72	30	34	34	.5	1	26	22	20	11.4	1	LGC	26	22	20	11.4	0		482	63	.	420	.	.
75	30	28	30	.5	2	26	22	22	12.6	1	TM	24	22	20	10.6	1	TM	500	65	.	.	.	.
78	26	26	26	.5	1	26	22	20	11.4	1	TM	26	24	22	13.7	2	TM	3017	67	.	.	.	.
81	28	28	30	.5	2	22	18	14	5.5	1	TM	34	26	24	21.2	2	MGC	5097	67	.	.	.	.
84	28	30	30	1	2	26	20	16	8.3	1	LR	36	32	26	30.0	2	MGC	5695	67	.	413	.	.
87	28	28	30	1	2	24	20	16	7.7	1	LGC	34	28	26	24.8	2	MGC	6091	65	.	.	.	.
90	28	28	28	1	2	24	22	20	10.6	1	LGC	34	28	26	24.8	2	MGC	6953	64	.	420	91	4.0

TABLE A-17 CONTINUED POSTPARTUM DATA

COW NUMBER=11 TREATMENT=22 BREED=3

DAY	CVX	LHO	RHO	UTL	TON	LOL	LOH	LOW	LEFT OVARY	LST	LSL	ROL	ROH	ROW	RIGHT OVARY	RST	RSL	PROGESTERONE	GLUCOSE	LH	COW WT	CF WT	CON SCORE
1	.	.	.	.	.	.	.	.	.	.		.	.	.	.	.		557	81	0.15	395	30	4.0
2	.	.	.	.	.	.	.	.	.	.		.	.	.	.	.		402	67	0.10	.	.	.
6	.	.	.	.	.	.	.	.	.	.		.	.	.	.	.		132	63	0.00	.	.	.
9	.	.	.	.	.	.	.	.	.	.		.	.	.	.	.		246	77	0.20	.	.	.
12	40	36	38	.5	1	20	16	14	4.5	0		16	14	12	2.7	0		184	63	0.05	397	.	.
15	32	32	30	.5	1	22	18	16	6.3	0		20	16	14	4.5	0		186	67	0.10	.	.	.
18	30	30	28	.5	1	20	16	14	4.5	0		18	14	12	3.0	0		126	67	0.30	.	.	.
21	30	30	34	.5	1	20	20	16	6.4	0		18	16	12	3.5	0		132	70	0.05	.	.	.
24	28	28	30	.5	1	22	18	14	5.5	0		22	16	14	4.9	0		131	38	0.20	395	.	.
27	30	30	32	.5	1	22	18	14	5.5	0		24	22	18	9.5	0		172	70	0.18	.	.	.
30	24	26	26	.5	1	22	16	14	4.9	0		22	18	14	5.5	0		264	68	0.83	.	.	.
33	24	24	28	.5	1	20	16	14	4.5	0		24	18	14	6.0	0		827	80	0.30	.	.	.
36	26	28	30	.5	1	16	14	12	2.7	0		20	20	18	7.2	1	LM	4528	68	0.23	400	.	.
39	28	32	34	.5	2	20	14	12	3.4	0		24	20	18	8.6	1	TM	563	66	0.35	.	.	4.7
42	26	26	30	.5	2	16	12	10	1.9	0		24	20	18	8.6	0		1597	65	.	.	.	.
45	24	26	24	.5	1	22	16	14	4.9	0		26	24	22	13.7	0		4844	65	.	.	.	.
48	24	24	28	1	1	20	14	12	3.4	0		22	18	16	6.3	1	TM	4304	65	.	404	.	.
51	24	26	28	1	1	16	14	10	2.2	0		24	22	20	10.6	0		9526	66	.	.	.	.
54	28	28	30	1	1	22	18	16	6.3	0		24	24	20	11.5	1	MGC	6413	63	.	.	.	.
57	24	24	28	.5	1	22	20	16	7.0	0		24	18	14	6.0	0		546	70	.	.	.	.
60	30	32	36	.5	2	20	16	14	4.5	0		22	18	16	6.3	1	LM	195	68	.	397	.	.
63	26	28	26	.5	1	26	18	16	7.5	0		20	16	12	3.8	0		1603	69	.	.	.	.
66	24	26	26	1	1	26	22	20	11.4	2	LGC	22	18	14	5.5	0		3810	64	.	.	.	.
69	26	26	26	1	1	28	24	20	13.4	2	LGC	20	18	14	5.0	0		6057	68	.	.	.	.
72	26	28	28	1	1	28	24	22	14.8	2	LGC	22	18	14	5.5	0		5522	65	.	424	.	.
75	28	30	34	.5	2	26	22	18	10.3	2	LGC	20	16	14	4.5	0		6718	65	.	.	.	.
78	24	26	26	.5	2	24	20	16	7.7	2	LGC	22	16	14	4.9	0		6120	72	.	406	70	5.0

TABLE A-17 CONTINUED POSTPARTUM DATA

COW NUMBER=12 TREATMENT=11 BREED=1

D A Y	C V X	L H O	R H O	U T L	T O N	L O L	L O H	L O W	LEFT OVARY	L S T	L S L	R O L	R O H	R O W	RIGHT OVARY	R S T	R S L	PRO GESTE RONE	GLU CO SE	LH	COW WT	CF WT	CON SCO RE
0	.	.	.	.	.	.	.	.	.	.		.	.	.	.	.		417	62	0.05	395	36	7.0
3	.	.	.	.	.	.	.	.	.	.		.	.	.	.	.		263	66	0.00	.	.	.
6	.	.	.	.	.	.	.	.	.	.		.	.	.	.	.		483	71	0.00	.	.	.
9	.	.	.	.	.	.	.	.	.	.		.	.	.	.	.		428	66	0.15	.	.	.
12	38	30	22	.0	1	24	14	12	4.0	0		26	16	12	5.0	0		516	68	0.05	.	.	.
15	38	34	36	.0	1	24	20	16	7.7	0		24	18	14	6.0	0		181	72	0.05	409	.	.
18	36	36	32	.0	1	22	16	12	4.2	0		22	18	14	5.5	0		2208	86	0.15	.	.	.
21	32	34	32	.5	1	20	18	16	5.8	0		32	28	20	17.9	1	LM	241	64	0.10	.	.	.
24	28	26	24	1	1	26	18	16	7.5	0		24	20	18	8.6	1	RGC	46	73	0.05	.	.	.
27	24	24	26	.5	1	24	22	18	9.5	0		24	20	18	8.6	1	LGC	126	74	0.05	403	.	.
30	28	30	32	1	1	24	20	20	9.6	0		26	20	18	9.4	1	LM	93	70	0.05	.	.	.
33	26	28	26	.5	1	22	18	16	6.3	0		24	20	16	7.7	0		38	68	0.00	.	.	.
36	26	28	26	1	1	24	22	16	8.4	1	TM	26	20	16	8.3	1	LM	230	73	0.10	.	.	.
39	24	28	24	1	1	18	14	12	3.0	0		24	20	18	8.6	0		219	76	0.20	397	.	6.0
42	.	.	.	.	.	.	.	.	.	.		.	.	.	.	.		175	.	.	.	.	.
45	26	32	32	1	2	26	20	16	8.3	0		24	18	14	6.0	1	TM	434	69	.	.	.	.
48	30	34	32	1	2	24	16	12	4.6	0		26	18	16	7.5	1	LM	324	67	.	.	.	.
51	24	28	26	1	2	22	20	16	7.0	1	LM	24	20	18	8.6	1	TM	346	62	.	381	.	.
54	28	32	30	1	1	22	18	14	5.5	1	RGC	24	20	18	8.6	0		252	78	.	.	.	.
57	28	32	32	.5	1	20	16	14	4.5	0		24	20	16	7.7	1	TM	873	70	.	.	.	.
60	24	26	26	1	2	24	18	16	6.9	1	LR	24	22	20	10.6	1	TM	533	68	.	.	.	.
63	26	28	26	.5	1	20	16	14	4.5	0		26	24	20	12.5	1	LM	2494	70	.	402	.	.
66	23	23	23	.5	1	22	18	16	6.3	0		30	24	22	15.8	1	LGC	7559	65	.	.	.	.
69	26	28	28	1	1	24	20	18	8.6	1	MGC	36	30	28	30.2	1	TM	7400	68	.	.	.	.
72	26	26	28	1	1	24	20	18	8.6	1	LM	32	28	24	21.5	1	LL	9389	76	.	.	.	.
75	28	30	28	1	1	24	20	18	8.6	1	TM	34	30	24	24.5	1	LGC	4516	69	.	422	89	7.7

TABLE A-17 CONTINUED POSTPARTUM DATA

COW NUMBER=13 TREATMENT=12 BREED=3

D A Y	C V X	L H O	R H O	U T L	T O N	L O L	L O H	L O W	LEFT OVARY	L S T	L S L	R O L	R O H	R O W	RIGHT OVARY	R S T	R S L	PRO GESTE RCNE	GLU CO SE	LH	COW WT	CF WT	CON SCO RE
0	.	.	.	.	.	.	.	.	.	.		.	.	.	.	.		631	73	0.10	320	32	5.0
2	.	.	.	.	.	.	.	.	.	.		.	.	.	.	.		842	66	0.30	.	.	.
5	.	.	.	.	.	.	.	.	.	.		.	.	.	.	.		1705	68	0.00	.	.	.
8	.	.	.	.	.	.	.	.	.	.		.	.	.	.	.		1763	86	0.10	.	.	.
11	34	34	36	.0	1	16	12	12	2.3	0		18	14	12	3.0	0		1963	84	0.00	304	.	.
14	34	36	34	.5	1	24	20	16	7.7	0		26	20	16	8.3	0		794	85	0.00	.	.	.
17	30	30	34	.5	1	18	12	10	2.2	0		24	16	12	4.6	0		663	70	0.00	.	.	.
20	30	28	32	.5	1	20	14	12	3.4	0		22	16	14	4.9	0		910	74	0.00	.	.	.
23	26	24	28	1	1	20	14	12	3.4	0		24	20	18	8.6	1	LM	905	88	0.00	295	.	.
26	24	26	28	.5	1	20	16	14	4.5	0		22	18	14	5.5	0		1810	81	0.00	.	.	.
29	24	26	26	.5	1	16	12	10	1.9	0		22	18	14	5.5	0		1742	84	0.00	.	.	.
32	30	30	32	.5	1	20	14	12	3.4	0		24	18	14	6.0	0		2120	84	0.00	.	.	.
35	26	26	30	.5	1	22	18	14	5.5	0		22	16	14	4.9	0		1321	68	0.00	304	.	.
38	24	24	26	1	1	20	14	12	3.4	0		24	20	18	8.6	0		1073	55	0.00	.	.	3.0
41	24	24	26	1	1	18	14	12	3.0	0		22	16	14	4.9	0		563	91	.	.	.	.
44	24	24	24	1	1	22	16	14	4.9	1	TM	20	16	14	4.5	0		4111	77	.	.	.	.
47	24	26	28	1	1	22	16	14	4.9	1	TM	20	16	12	3.8	0		3273	84	.	.	.	.
50	24	24	26	1	1	22	18	14	5.5	0		22	18	14	5.5	0		1464	86	.	311	.	.
53	26	24	26	1	1	22	18	14	5.5	0		24	16	14	5.4	0		884	82	.	.	.	.
56	26	26	28	1	1	22	18	14	5.5	0		24	18	14	6.0	0		505	71	.	.	.	.
59	26	24	26	.5	1	20	16	12	3.8	0		24	20	18	8.6	0		615	72	.	.	.	.
62	24	24	26	1	1	22	18	16	6.3	1	LGC	24	20	16	7.7	1	LM	2263	72	.	316	.	.
65	26	24	26	1	1	20	16	14	4.5	0		22	20	16	7.0	0		510	74	.	.	.	.
68	26	24	28	1	1	22	18	14	5.5	0		24	22	20	10.6	1	LM	2121	78	.	.	.	.
71	26	24	26	1	1	22	16	14	4.9	0		22	18	14	5.5	0		1757	80	.	.	.	.
74	24	24	24	1	1	22	16	14	4.9	1	LM	24	18	16	6.9	0		284	75	.	329	.	.
77	28	26	28	1	1	20	14	12	3.4	0		24	18	16	6.9	1	TM	442	73	.	.	.	.
80	28	24	26	1	1	20	16	14	4.5	0		24	20	18	8.6	0		905	80	.	.	.	.
83	28	28	30	1	1	24	20	16	7.7	1	TR	22	18	14	5.5	0		2563	70	.	.	.	.
86	28	28	30	1	2	24	22	16	8.4	1	LGC	24	18	16	6.9	0		436	68	.	338	.	.
89	28	28	30	.5	2	24	16	14	5.4	2	TM	22	16	14	4.9	0		2042	74	.	331	61	5.3

TABLE A-17 CONTINUED POSTPARTUM DATA

COW NUMBER=14 TREATMENT=22 BREED=3

DAY	CVX	LHO	RHO	UTL	TON	LOL	LOH	LOW	LEFT OVARY	LST	LSL	ROL	ROH	ROW	RIGHT OVARY	RST	RSL	PROGESTERONE	GLUCOSE	LH	COW WT	CF WT	CON SCORE
0	.	.	.	.	.	.	.	.	.	.		.	.	.	.	.		405	73	0.05	372	27	6.0
3	.	.	.	.	.	.	.	.	.	.		.	.	.	.	.		477	74	0.13	.	.	.
6	.	.	.	.	.	.	.	.	.	.		.	.	.	.	.		1834	78	0.05	.	.	.
9	48	52	54	.0	1	26	22	16	9.2	0		22	18	14	5.5	0		922	68	0.10	.	.	.
12	38	48	46	.0	1	22	20	16	7.0	0		20	16	14	4.5	0		116	64	0.20	356	.	.
15	34	38	36	.5	1	22	20	16	7.0	0		24	18	16	6.9	0		805	67	0.15	.	.	.
18	28	32	28	.5	1	22	16	12	4.2	0		22	18	16	6.3	0		611	58	0.20	.	.	.
21	32	32	30	1	1	20	16	12	3.8	0		22	20	16	7.0	0		627	63	0.13	.	.	.
24	28	30	28	1	1	24	18	16	6.9	0		20	18	16	5.8	0		411	68	0.05	345	.	.
27	26	26	28	1	1	24	16	14	5.4	0		22	18	16	6.3	0		672	70	0.00	.	.	.
30	28	30	28	1	1	22	14	12	3.7	0		24	20	16	7.7	0		888	67	0.18	.	.	.
33	26	26	28	1	1	20	16	12	3.8	0		24	20	18	8.6	0		516	56	0.15	.	.	.
36	26	26	28	1	1	22	16	14	4.9	0		24	18	16	6.9	0		738	76	0.10	363	.	.
39	28	30	28	1	1	24	18	16	6.9	0		22	18	14	5.5	1	LM	439	61	0.10	.	.	5.0
42	30	34	32	1	1	22	16	14	4.9	0		24	20	16	7.7	1	LR	492	78	.	.	.	.
45	26	28	26	1	1	22	16	14	4.9	0		24	20	16	7.7	1	MGC	544	68	.	.	.	.
48	28	28	28	1	1	24	20	16	7.7	0		22	18	16	6.3	1	TM	288	63	.	336	.	.
51	28	30	28	1	1	20	14	12	3.4	0		22	16	14	4.9	1	LL	46	60	.	.	.	.
54	28	28	28	1	1	22	18	16	6.3	1	TM	24	20	16	7.7	0		175	66	.	.	.	.
57	28	26	26	1	1	22	14	12	3.7	0		22	18	14	5.5	1	LL	105	61	.	.	.	.
60	28	26	28	1	1	24	20	16	7.7	1	TM	24	20	16	7.7	0		266	59	.	393	.	.
63	26	28	28	1	2	24	16	14	5.4	0		28	24	20	13.4	1	LGC	2266	63	.	.	.	.
66	30	34	32	1	2	20	14	12	3.4	0		26	18	16	7.5	1	LGC	77	63	.	.	.	.
69	32	32	30	1	2	22	16	14	4.9	1	LM	24	20	18	8.6	1	MGC	316	60	.	.	.	.
72	26	28	28	1	2	22	16	14	4.9	0		26	24	20	12.5	2	MGC	1966	79	.	397	.	.
75	28	28	28	1	2	24	16	14	5.4	1	TM	24	20	18	8.6	2	RGC	2671	56	.	.	.	.
78	26	26	28	1	2	22	18	16	6.3	0		28	24	20	13.4	2	RGC	4194	69	.	.	.	.
81	28	30	28	1	2	22	18	14	5.5	1	LM	30	24	20	14.4	2	MGC	5610	55	.	.	.	.
84	26	26	28	1	2	22	16	12	4.2	0		28	26	22	16.0	2	MGC	3999	55	.	400	.	.
87	26	28	26	1	2	22	18	14	5.5	0		26	22	18	10.3	2	MGC	0	59	.	.	.	.
90	26	26	26	1	2	22	16	14	4.9	2	PM	22	18	14	5.5	0		203	41	.	413	86	6.7

TABLE A-17 CONTINUED POSTPARTUM DATA

COW NUMBER=15 TREATMENT=12 BREED=1

D A Y	C V X	L H O	R H O	U T L	T O N	L O L	L O H	L O W	LEFT OVARY	L S T	L S L	R O L	R O H	R O W	RIGHT OVARY	R S T	R S L	PRO GESTE RONE	GLU CO SE	LH	COW WT	CF WT	CON SCO RE
1	.	.	.	.	.	.	.	.	.	.		.	.	.	.	.		426	83	0.00	400	32	6.0
4	.	.	.	.	.	.	.	.	.	.		.	.	.	.	.		636	96	0.00	.	.	.
7	.	.	.	.	.	.	.	.	.	.		.	.	.	.	.		547	78	0.00	.	.	.
10	48	44	48	.0	1	20	14	12	3.4	0		22	18	14	5.5	0		663	89	0.05	386	.	.
13	40	38	38	.0	1	22	16	14	4.9	0		22	18	14	5.5	0		615	.	0.00	.	.	.
16	32	34	36	.0	1	22	16	12	4.2	0		22	16	14	4.9	0		710	77	0.00	.	.	.
19	34	34	38	.0	1	22	16	14	4.9	0		22	16	14	4.9	0		468	86	0.15	.	.	.
22	36	36	38	.0	1	20	16	12	3.8	0		20	16	14	4.5	0		705	80	0.00	372	.	.
25	26	26	28	.0	1	20	16	14	4.5	0		22	18	16	6.3	0		410	79	0.00	.	.	.
28	28	26	28	1	1	22	18	14	5.5	1	MGC	20	16	14	4.5	0		2273	118	0.00	.	.	.
31	28	28	34	.5	1	20	16	12	3.8	1	TM	22	18	14	5.5	0		621	89	0.00	.	.	.
34	32	34	38	.5	1	22	16	14	4.9	1	LGC	24	18	14	6.0	0		715	91	0.00	370	.	.
37	24	24	26	1	1	22	18	14	5.5	1	RGC	24	20	16	7.7	0		452	87	0.00	.	.	6.3
40	26	28	28	.5	1	20	16	14	4.5	1	TM	20	16	12	3.8	0		173	103	.	.	.	.
43	26	26	28	1	1	22	14	12	3.7	0		22	18	16	6.3	0		489	82	.	.	.	.
46	28	32	30	.5	1	20	16	14	4.5	1	LM	24	22	18	9.5	0		526	85	.	.	.	.
49	28	28	30	.5	1	22	20	18	7.9	0		22	18	16	6.3	0		73	74	.	356	.	.
52	26	24	28	.5	1	22	16	14	4.9	0		24	20	16	7.7	0		278	75	.	.	.	.
55	26	26	28	.0	1	24	18	14	6.0	0		20	16	14	4.5	0		263	77	.	.	.	.
58	28	26	26	1	1	20	16	12	3.8	0		24	16	14	5.4	0		363	82	.	.	.	.
61	28	26	28	.5	1	22	16	14	4.9	0		22	20	16	7.0	0		73	78	.	375	.	.
64	28	26	28	1	1	20	16	12	3.8	0		20	18	14	5.0	0		857	72	.	.	.	.
67	26	26	28	1	1	24	20	16	7.7	0		22	18	16	6.3	0		194	89	.	.	.	.
70	26	24	26	1	1	20	14	12	3.4	0		24	18	14	6.0	0		179	87	.	.	.	.
73	26	26	26	1	1	20	14	12	3.4	0		22	18	14	5.5	0		742	107	.	372	.	.
76	24	24	26	1	1	18	12	10	2.2	0		20	18	14	5.0	1	TM	236	77	.	.	.	.
79	24	24	26	1	1	22	16	14	4.9	1	TM	24	18	16	6.9	1	TM	189	76	.	.	.	.
82	28	26	30	1	1	20	14	12	3.4	1	TL	22	20	16	7.0	1	TM	626	75	.	.	.	.
85	26	26	28	1	1	22	18	16	6.3	1	TM	24	20	16	7.7	1	TM	2063	83	.	377	.	.
88	26	26	28	1	2	20	16	12	3.8	1	LM	24	20	18	8.6	1	MGC	2068	75	.	.	.	.
91	28	30	30	1	2	20	16	14	4.5	1	MGC	24	20	16	7.7	1	TM	315	90	.	365	91	6.3

TABLE A-17 CONTINUED POSTPARTUM DATA

COW NUMBER=16 TREATMENT=12 BREED=1

DAY	CVX	LHU	RHU	UTL	TON	LOL	LOH	LOW	LEFT OVARY	LST	LSL	ROL	ROH	ROW	RIGHT OVARY	RST	RSL	PROGESTERONE	GLUCOSE	LH	COW WT	CF WT	CON SCORE
1	.	.	.	.	.	.	.	.	.	.		.	.	.	.	.		649	82	0.20	327	41	6.7
4	.	.	.	.	.	.	.	.	.	.		.	.	.	.	.		338	88	0.00	.	.	.
7	.	.	.	.	.	.	.	.	.	.		.	.	.	.	.		433	67	0.23	.	.	.
10	50	54	56	.0	1	24	16	14	5.4	0		22	18	14	5.5	0		316	65	0.30	331	.	.
13	36	38	34	.0	1	26	20	16	8.3	0		26	20	18	9.4	0		799	73	0.33	.	.	.
16	34	38	36	.0	1	22	18	16	6.3	0		20	16	12	3.8	0		72	67	0.33	.	.	.
19	32	38	34	.5	1	26	20	16	8.3	0		24	16	14	5.4	0		4092	76	0.30	.	.	.
22	30	36	32	.5	1	24	20	16	7.7	0		26	18	14	6.6	0		705	75	0.38	343	.	.
25	30	34	32	.5	1	22	16	14	4.9	0		24	20	16	7.7	0		2524	76	0.38	.	.	.
28	28	30	26	.5	1	24	14	12	4.0	1	MGC	26	20	16	8.3	0		1459	77	0.55	.	.	.
31	24	26	24	1	1	24	16	14	5.4	0		22	18	16	6.3	0		544	77	0.25	.	.	.
34	24	28	26	.5	2	24	18	14	6.0	0		26	22	20	11.4	0		905	68	0.33	340	.	.
37	26	28	24	.5	1	24	18	14	6.0	0		22	16	14	4.9	0		816	67	0.30	.	.	.
40	28	32	34	.5	1	22	18	14	5.5	1	LGC	24	18	14	6.0	0		544	65	0.23	.	.	5.0
43	28	32	30	.5	1	22	18	16	6.3	0		24	20	16	7.7	1	LM	511	88	.	.	.	.
46	28	32	30	.0	1	22	20	18	7.9	0		26	20	16	8.3	0		538	68	.	345	.	.
49	28	28	30	1	1	22	16	14	4.9	0		28	22	20	12.3	1	LM	566	74	.	.	.	.
52	26	28	28	1	1	22	18	14	5.5	1	LM	28	22	20	12.3	1	TM	305	75	.	.	.	.
55	26	28	26	1	1	22	18	14	5.5	0		26	20	16	8.3	1	LGC	211	70	.	.	.	.
58	26	28	26	1	1	20	16	14	4.5	1	MGC	26	22	20	11.4	0		483	63	.	354	.	.
61	28	28	30	1	1	22	16	14	4.9	0		26	20	18	9.4	1	TM	977	76	.	.	.	.
64	28	30	28	.5	1	24	20	16	7.7	1	LM	24	22	18	9.5	1	TM	522	69	.	.	.	.
67	30	32	30	1	2	22	18	14	5.5	0		26	24	20	12.5	1	LM	388	68	.	.	.	.
70	28	30	28	1	2	22	16	14	4.9	1	LR	22	18	16	6.3	1	LM	599	67	.	338	.	.
73	28	28	26	1	2	22	16	14	4.9	1	LR	26	24	18	11.2	2	MGC	2599	72	.	.	.	.
76	28	28	28	1	2	26	20	16	8.3	1	TM	34	28	24	22.8	2	MGC	5916	64	.	.	.	.
79	28	30	28	1	2	22	18	14	5.5	1	TM	30	26	24	18.7	2	MGC	6427	65	.	.	.	.
82	28	28	28	1	2	22	20	16	7.0	1	TM	26	24	20	12.5	2	MGC	7271	71	.	350	.	.
85	28	28	26	1	2	22	18	14	5.5	1	LL	26	22	20	11.4	2	MGC	227	67	.	.	.	.
88	28	30	30	1	2	22	18	14	5.5	1	TM	24	20	18	8.6	1	LR	1150	67	.	.	.	.
91	26	28	26	1	2	22	18	16	6.3	1	TM	22	16	14	4.9	1	MGC	1455	67	.	368	95	7.0

TABLE A-17 CONTINUED POSTPARTUM DATA

COW NUMBER=17 TREATMENT=22 BREED=2

DAY	CVX	LHO	RHO	UTL	TON	LOL	LOH	LOW	LEFT OVARY	LST	LSL	ROL	ROH	ROW	RIGHT OVARY	RST	RSL	PRO GESTE RONE	GLU CO SE	LH	COW WT	CF WT	CON SCO RE
0	.	.	.	.	.	.	.	.	.	.		.	.	.	.	.		294	94	0.05	363	43	6.0
3	.	.	.	.	.	.	.	.	.	.		.	.	.	.	.		300	61	0.00	.	.	.
6	.	.	.	.	.	.	.	.	.	.		.	.	.	.	.		452	69	0.00	.	.	.
9	48	50	50	.0	1	22	14	12	3.7	0		26	20	16	8.3	0		389	73	0.00	.	.	.
12	46	40	42	.0	1	24	14	12	4.0	0		26	16	14	5.8	0		384	59	0.00	350	.	.
15	36	32	34	.0	1	22	16	12	4.2	0		26	20	16	8.3	0		826	97	0.00	.	.	.
18	42	38	38	.5	1	22	18	14	5.5	0		26	20	18	9.4	0		352	71	0.00	.	.	.
21	32	32	30	1	1	20	16	14	4.5	0		18	16	14	4.0	0		678	75	0.00	.	.	.
24	26	24	26	1	1	22	18	16	6.3	0		26	20	16	8.3	0		1084	69	0.00	343	.	.
28	30	30	32	1	1	26	20	18	9.4	1	LM	22	18	14	5.5	0		452	66	0.00	.	.	.
30	24	24	26	1	1	22	18	14	5.5	0		24	18	16	6.9	0		589	82	0.00	.	.	.
33	24	26	24	1	1	24	18	14	6.0	0		24	20	16	7.7	0		1036	75	0.13	.	.	.
36	24	24	26	1	1	22	20	16	7.0	0		24	18	14	6.0	0		431	66	0.05	331	.	.
39	26	26	28	.5	1	20	16	12	3.8	0		24	20	18	8.6	0		2226	95	0.05	.	.	4.0
42	28	30	28	.5	2	22	16	12	4.2	0		22	16	14	4.9	1	RGC	189	.	.	.	.	.
45	24	28	26	1	1	22	16	14	4.9	0		24	20	18	8.6	1	LGC	915	80	.	.	.	.
48	26	28	26	1	1	20	12	10	2.4	0		22	16	14	4.9	0		836	61	.	322	.	.
51	24	24	24	1	1	22	12	10	2.6	0		24	18	16	6.9	0		589	82	.	.	.	.
54	26	28	26	1	1	20	16	12	3.8	0		22	20	16	7.0	0		473	74	.	.	.	.
57	24	26	26	1	1	22	16	14	4.9	0		22	16	12	4.2	0		342	75	.	.	.	.
60	24	26	26	1	1	20	16	14	4.5	0		22	18	14	5.5	0		707	73	.	322	.	.
63	24	28	26	1	1	24	18	14	6.0	0		20	16	14	4.5	0		604	66	.	.	.	.
66	26	26	30	1	1	20	14	12	3.4	0		22	20	16	7.0	0		647	79	.	.	.	.
69	24	24	26	1	1	20	14	12	3.4	0		24	18	14	6.0	0		789	79	.	.	.	.
72	24	26	24	1	1	24	16	12	4.6	0		24	20	18	8.6	1	TM	263	80	.	336	.	.
75	28	26	28	1	1	24	18	16	6.9	1	TM	22	18	14	5.5	0		231	77	.	.	.	.
78	26	28	28	.5	1	24	18	16	6.9	1	TM	22	18	16	6.3	1	TM	910	80	.	.	.	.
81	28	28	26	.5	1	22	14	12	3.7	0		22	16	14	4.9	0		1015	82	.	.	.	.
84	28	28	30	1	1	22	14	12	3.7	0		26	22	18	10.3	1	LM	363	88	.	352	.	.
87	26	26	28	1	1	22	14	12	3.7	1	TM	24	20	16	7.7	1	LR	600	80	.	.	.	.
90	28	28	28	1	1	22	16	14	4.9	0		24	18	16	6.9	1	LGC	2015	72	.	350	98	5.7

TABLE A-17 CONTINUED POSTPARTUM DATA

COW NUMBER=18 TREATMENT=21 BREED=3

DAY	CVX	LHO	RHO	UTL	TON	LOL	LOH	LOW	LEFT OVARY	LST	LSL	ROL	ROH	ROW	RIGHT OVARY	RST	RSL	PROGESTERONE	GLUCOSE	LH	COW WT	CF WT	CON SCORE
1	.	.	.	.	.	.	.	.	.	.		.	.	.	.	.		387	79	0.00	322	34	5.0
4	.	.	.	.	.	.	.	.	.	.		.	.	.	.	.		294	65	0.05	.	.	.
7	.	.	.	.	.	.	.	.	.	.		.	.	.	.	.		226	73	0.00	.	.	.
10	46	44	48	.0	1	22	18	14	5.5	0		22	18	16	6.3	0		221	68	0.00	322	.	.
13	34	36	36	.5	1	24	14	10	3.4	0		26	14	10	3.6	0		452	87	0.00	.	.	.
16	30	32	34	1	1	22	16	12	4.2	0		24	18	14	6.0	0		263	72	0.00	.	.	.
19	28	30	28	.5	1	22	18	14	5.5	0		20	14	10	2.8	0		358	72	0.00	.	.	.
22	30	32	34	.5	1	26	20	16	8.3	0		22	16	14	4.9	0		1694	83	0.00	322	.	.
25	24	24	26	1	1	22	16	14	4.9	0		22	16	12	4.2	0		1615	85	0.00	.	.	.
28	28	30	34	1	1	20	16	12	3.8	1	MGC	18	14	12	3.0	0		1442	73	0.00	.	.	.
31	30	30	32	.5	1	20	14	10	2.8	0		24	16	12	4.6	0		122	90	0.00	.	.	.
34	24	26	26	1	1	20	12	10	2.4	0		26	20	16	8.3	0		684	78	0.00	320	.	.
37	26	26	30	.5	1	18	12	10	2.2	0		22	18	14	5.5	0		700	81	0.00	.	.	.
40	24	24	26	1	2	22	16	14	4.9	0		24	18	14	6.0	0		526	72	0.00	.	.	6.3
43	26	26	30	1	1	20	14	10	2.8	0		20	16	14	4.5	0		273	84	.	.	.	.
46	26	26	28	1	1	22	16	12	4.2	0		24	16	14	5.4	0		558	73	.	309	.	.
49	28	26	30	.5	1	22	18	14	5.5	0		24	20	16	7.7	0		336	72	.	.	.	.
52	26	26	28	1	1	20	16	14	4.5	0		20	14	12	3.4	0		226	75	.	.	.	.
55	26	24	26	1	1	22	16	14	4.9	0		22	16	14	4.9	0		251	59	.	.	.	.
58	26	26	26	1	1	22	16	14	4.9	0		24	20	16	7.7	0		251	78	.	334	.	.
61	26	26	28	1	1	20	14	12	3.4	0		22	18	14	5.5	0		247	72	.	.	.	.
64	24	24	24	1	1	20	16	14	4.5	1	TM	24	20	16	7.7	1	RGC	289	75	.	.	.	.
67	24	24	26	1	1	22	16	14	4.9	0		24	20	16	7.7	0		257	78	.	.	.	.
70	26	24	26	1	1	20	16	14	4.5	1	TM	20	16	14	4.5	0		94	76	.	331	.	.
73	26	28	26	1	1	18	14	12	3.0	0		20	18	14	5.0	0		231	76	.	.	.	.
76	28	28	28	1	1	22	18	16	6.3	1	RGC	20	16	14	4.5	1	LM	1415	72	.	.	.	.
79	28	28	30	1	2	20	14	14	3.9	0		20	16	14	4.5	1	MGC	437	73	.	.	.	.
82	24	24	24	1	2	20	14	12	3.4	0		24	14	12	4.0	0		3036	70	.	334	.	.
85	26	26	26	1	2	20	16	12	3.8	1	TM	24	20	16	7.7	2	MGC	2968	75	.	.	.	.
88	26	26	28	1	2	22	16	14	4.9	0		26	18	16	7.5	2	MGC	4973	78	.	.	.	.
91	26	28	28	1	2	22	16	14	4.9	0		28	24	20	13.4	2	MGC	4615	76	.	340	91	5.0

TABLE A-17 CONTINUED POSTPARTUM DATA

COW NUMBER=19 TREATMENT=21 BREED=1

DAY	CVX	LHO	RHO	UTL	TON	LOL	LOH	LOW	LEFT OVARY	LST	LSL	ROL	ROH	ROW	RIGHT OVARY	RST	RSL	PROGESTERONE	GLUCOSE	LH	COW WT	CF WT	CON SCORE
1	.	.	.	.	.	.	.	.	.	.		.	.	.	.	.		349	67	0.13	372	48	3.0
3	.	.	.	.	.	.	.	.	.	.		.	.	.	.	.		1133	71	0.20	.	.	.
6	.	.	.	.	.	.	.	.	.	.		.	.	.	.	.		994	64	0.05	.	.	.
9	50	52	56	.0	1	22	18	14	5.5	0		18	16	12	3.5	0		677	60	0.00	.	.	.
12	36	36	34	.5	1	22	16	14	4.9	0		24	18	14	6.0	0		1249	61	0.00	350	.	.
15	32	34	36	.5	1	22	14	12	3.7	0		24	22	18	9.5	0		2194	74	0.10	.	.	.
18	30	32	34	.0	1	20	14	12	3.4	0		24	20	16	7.7	0		622	92	0.15	.	.	.
21	32	32	36	.5	1	20	14	12	3.4	0		22	16	14	4.9	0		904	96	0.33	.	.	.
24	26	30	28	1	1	20	14	12	3.4	0		22	18	14	5.5	0		4232	67	0.05	347	.	.
27	26	28	30	1	1	20	16	12	3.8	0		22	18	14	5.5	0		1283	68	0.05	.	.	.
30	26	26	32	1	1	26	18	14	6.6	0		24	20	16	7.7	0		966	80	0.05	.	.	.
33	24	24	26	1	1	22	16	14	4.9	0		22	18	16	6.3	1	LL	1483	84	0.10	.	.	.
36	26	28	30	.5	1	22	16	12	4.2	0		24	20	16	7.7	0		2433	60	0.00	.	.	.
39	26	24	28	1	1	22	16	14	4.9	0		24	20	16	7.7	0		816	74	0.05	.	.	.
42	26	28	30	1	1	22	18	16	6.3	1	LR	26	22	20	11.4	0		772	57	0.05	.	.	3.0
45	28	26	30	1	1	24	18	14	6.0	0		24	20	16	7.7	1	LM	277	78	.	.	.	.
48	24	26	26	1	1	22	18	14	5.5	0		26	20	16	8.3	1	TM	250	74	.	343	.	.
51	28	30	32	1	1	24	18	14	6.0	0		24	20	16	7.7	1	TM	366	80	.	.	.	.
54	26	26	30	1	1	26	18	16	7.5	0		28	24	20	13.4	1	LR	149	54	.	.	.	.
57	26	26	28	1	1	22	18	16	6.3	1	LGC	20	18	14	5.0	0		183	64	.	.	.	.
60	26	26	28	1	1	22	16	14	4.9	0		24	20	18	8.6	1	LGC	166	67	.	372	.	.
63	26	26	30	1	1	22	18	14	5.5	1	TM	24	20	18	8.6	0		272	68	.	.	.	.
66	26	26	28	1	1	24	18	16	6.9	0		26	22	18	10.3	1	LM	227	63	.	.	.	.
69	26	26	28	1	1	22	16	14	4.9	0		24	20	16	7.7	1	LM	372	64	.	.	.	.
72	24	26	26	1	1	22	18	16	6.3	1	MGC	24	20	16	7.7	1	LM	322	60	.	386	.	.
75	26	26	26	1	1	20	16	14	4.5	1	LM	24	20	14	6.7	1	LM	333	58	.	.	.	.
78	24	26	26	1	1	24	18	14	6.0	1	TM	32	24	18	13.8	1	TM	438	58	.	.	.	.
81	26	26	26	1	1	20	16	14	4.5	1	RGC	22	18	14	5.5	1	LM	227	62	.	.	.	.
84	26	26	28	1	1	22	16	14	4.9	1	TM	32	26	20	16.6	1	TM	250	64	.	377	.	.
87	24	24	24	1	1	22	16	14	4.9	1	LM	24	20	18	8.6	1	LR	372	54	.	.	.	.
90	26	26	26	1	1	22	16	14	4.9	1	MGC	24	20	16	7.7	1	LM	494	74	.	393	102	5.7

TABLE A-17 CONTINUED POSTPARTUM DATA

COW NUMBER=20 TREATMENT=11 BREED=1

DAY	CVX	LHO	RHC	UTL	TON	LOL	LOH	LOW	LEFT OVARY	LST	LSL	ROL	ROH	ROW	RIGHT OVARY	RST	RSL	PROGESTERONE	GLUCOSE	LH	COW WT	CF WT	CONSCORE
0	.	.	.	.	.	.	.	.	.	.		.	.	.	.	.		457	88	0.05	322	34	6.0
1	.	.	.	.	.	.	.	.	.	.		.	.	.	.	.		415	66	0.00	.	.	.
4	.	.	.	.	.	.	.	.	.	.		.	.	.	.	.		989	79	0.13	.	.	.
7	.	.	.	.	.	.	.	.	.	.		.	.	.	.	.		678	72	0.05	.	.	.
10	46	44	48	.0	1	26	22	18	10.3	0		26	20	16	8.3	0		957	72	0.05	.	.	.
13	36	40	36	.0	1	26	20	16	8.3	0		28	18	16	8.1	0		1194	78	0.00	329	.	.
16	34	36	38	.5	1	26	20	16	8.3	0		26	22	16	9.2	0		994	72	0.00	.	.	.
19	28	26	30	.5	1	24	20	16	7.7	0		24	20	18	8.6	0		678	74	0.05	.	.	.
22	30	30	36	.5	1	24	20	16	7.7	0		26	22	18	10.3	0		5705	106	0.13	.	.	.
25	30	32	36	.5	1	22	16	14	4.9	0		26	24	18	11.2	0		1326	75	0.13	322	.	.
28	30	34	32	.5	1	24	18	14	6.0	0		26	22	18	10.3	1	TM	352	65	0.10	.	.	.
31	26	28	26	.5	1	26	22	16	9.2	0		28	22	18	11.1	0		2452	75	0.25	.	.	.
34	26	26	28	.5	1	24	20	16	7.7	1	TM	26	22	18	10.3	0		1426	59	0.10	.	.	.
37	26	28	30	1	2	24	18	14	6.0	1	LM	24	20	18	8.6	0		831	72	0.00	325	.	4.7
40	28	30	34	.5	1	26	20	16	8.3	1	TM	24	20	16	7.7	0		115	57	.	.	.	.
43	32	32	34	.5	1	22	18	14	5.5	0		24	20	16	7.7	0		1310	71	.	.	.	.
46	26	26	28	1	1	22	18	16	6.3	0		26	24	20	12.5	1	RL	605	68	.	.	.	.
49	24	26	26	1	1	26	20	18	9.4	0		24	20	18	8.6	0		342	68	.	325	.	.
52	28	30	34	.5	1	26	20	18	9.4	0		26	22	20	11.4	1	LM	610	82	.	.	.	.
55	26	28	28	.5	1	26	22	20	11.4	1	TM	26	22	20	11.4	1	TM	447	79	.	.	.	.
58	26	28	26	1	1	24	20	18	8.6	1	MGC	26	22	20	11.4	1	TM	547	76	.	.	.	.
61	24	26	26	1	1	26	22	18	10.3	1	LM	26	24	20	12.5	0		1694	66	.	336	.	.
64	26	28	26	.5	1	22	16	14	4.9	1	MGC	26	24	20	12.5	1	TM	3368	80	.	.	.	.
67	26	28	26	1	2	22	20	16	7.0	1	LM	28	26	22	16.0	1	MGC	1747	74	.	.	.	.
70	30	32	32	1	1	24	20	16	7.7	1	TM	28	24	20	13.4	1	TM	415	80	.	.	.	.
73	28	30	28	1	2	22	20	18	7.9	1	LM	24	22	20	10.6	2	MGC	1184	76	.	340	.	.
76	26	28	28	.5	2	24	20	16	7.7	1	TM	24	22	18	9.5	2	MGC	3520	65	.	.	.	.
79	24	26	24	1	2	26	22	18	10.3	1	TM	34	26	24	21.2	2	LGC	7747	66	.	.	.	.
82	24	26	26	1	2	24	22	20	10.6	1	TM	34	26	22	19.4	2	LGC	5741	70	.	.	.	.
85	28	28	30	1	2	24	20	16	7.7	1	TM	26	22	16	9.2	2	LGC	8083	78	.	343	.	.
88	28	30	30	1	2	26	22	20	11.4	1	RGC	28	26	18	13.1	2	LGC	400	78	.	.	.	.
91	28	28	28	1	2	22	16	14	4.9	1	TM	24	20	16	7.7	0		442	109	.	350	91	6.3

TABLE A-17 CONTINUED POSTPARTUM DATA

COW NUMBER=21 TREATMENT=12 BREED=1

DAY	CVX	LHO	RHC	UTL	TON	LOL	LOH	LOW	LEFT OVARY	LST	LSL	ROL	ROH	ROW	RIGHT OVARY	RST	RSL	PROGESTERONE	GLUCOSE	LH	COW WT	CF WT	CONSCORE
0	.	.	.	.	.	.	.	.	.	.		.	.	.	.	.		493	77	0.30	436	34	6.0
3	.	.	.	.	.	.	.	.	.	.		.	.	.	.	.		475	61	0.05	.	.	.
6	.	.	.	.	.	.	.	.	.	.		.	.	.	.	.		337	60	0.00	.	.	.
9	60	50	55	.0	1	24	14	12	4.0	0		22	18	14	5.5	0		0	.	.	.	.	.
12	45	44	38	.0	1	18	12	10	2.2	0		28	22	18	11.1	0		431	67	0.28	420	.	.
15	40	30	34	.0	1	20	14	10	2.8	0		32	20	26	16.6	0		387	67	0.15	.	.	.
18	32	30	32	.5	1	16	12	10	1.9	0		34	26	20	17.7	0		597	65	0.25	.	.	.
21	34	32	36	.5	1	18	14	12	3.0	0		24	18	14	6.0	0		437	69	0.53	.	.	.
24	34	34	32	.0	1	22	16	14	4.9	0		32	22	18	12.7	0		318	77	0.53	424	.	.
27	32	32	34	.0	1	22	16	14	4.9	0		24	20	18	8.6	0		1731	71	0.00	.	.	.
30	30	32	34	.0	1	22	16	14	4.9	0		28	24	20	13.4	0		3775	72	0.05	.	.	.
33	32	36	32	.5	1	26	20	18	9.4	1	MGC	28	24	20	13.4	0		1281	71	.	.	.	.
36	28	26	28	.0	1	24	18	16	6.9	0		26	22	18	10.3	0		1337	71	0.20	413	.	.
39	28	28	32	.0	1	22	16	14	4.9	0		24	20	16	7.7	0		3787	69	0.10	.	.	6.0
42	28	30	34	.5	2	28	24	20	13.4	1	MGC	24	20	18	8.6	0		8750	.	.	.	.	.
45	26	26	28	.5	1	28	24	20	13.4	1	RGC	26	20	16	8.3	0		10176	76	.	.	.	.
48	24	26	26	.5	2	28	24	20	13.4	1	MGC	30	22	20	13.2	0		0	.	.	413	.	.
51	26	26	28	.5	1	22	20	20	8.8	1	MGC	24	20	18	8.6	0		3468	71	.	.	.	.
54	28	30	32	1	2	22	18	14	5.5	0		26	22	18	10.3	1	MGC	313	80	.	.	.	.
57	26	26	28	1	1	20	16	12	3.8	0		24	20	16	7.7	0		1640	65	.	.	.	.
60	24	26	30	.5	1	22	16	14	4.9	0		26	24	20	12.5	0		2618	63	.	434	.	.
63	30	32	34	.0	1	22	18	16	6.3	0		26	22	20	11.4	0		5543	65	.	.	.	.
66	26	28	28	.5	1	20	14	12	3.4	0		34	26	24	21.2	1	LGC	6400	65	.	.	.	.
69	30	34	32	.5	1	22	18	16	6.3	0		28	24	22	14.8	0		8294	71	.	.	.	.
72	28	28	30	.5	1	22	18	14	5.5	0		28	24	24	16.1	1	MGC	851	81	.	424	.	.
75	30	30	28	.5	2	20	16	14	4.5	1	LGC	24	20	16	7.7	1	TM	426	71	.	.	.	.
78	26	28	28	.5	1	22	18	14	5.5	0		26	22	20	11.4	0		1558	74	.	.	.	.
81	24	26	26	1	2	26	22	20	11.4	2	MGC	24	22	18	9.5	0		3412	74	.	.	.	.
84	26	26	26	.5	2	26	24	22	13.7	2	MGC	24	20	18	8.6	0		8000	74	.	445	.	.
87	24	26	26	.5	2	24	20	18	8.6	2	MGC	24	20	16	7.7	0		4234	69	.	.	.	.
90	28	28	28	.5	2	24	22	20	10.6	2	MGC	26	20	18	9.4	0		7104	62	.	443	73	6.0

TABLE A-17 CONTINUED POSTPARTUM DATA

COW NUMBER=22 TREATMENT=11 BREED=3

DAY	CVX	LHO	RHO	UTL	TON	LOL	LOH	LOW	LEFT OVARY	LST	LSL	ROL	ROH	ROW	RIGHT OVARY	RST	RSL	PROGESTERONE	GLUCOSE	LH	COW WT	CF WT	CON SCORE
1	.	.	.	.	.	.	.	.	.	.		.	.	.	.	.		219	65	0.10	372	50	5.0
4	.	.	.	.	.	.	.	.	.	.		.	.	.	.	.		532	70	0.10	.	.	.
7	.	.	.	.	.	.	.	.	.	.		.	.	.	.	.		626	83	0.15	.	.	.
10	.	.	.	.	.	.	.	.	.	.		.	.	.	.	.		857	76	0.15	.	.	.
13	42	46	48	.0	1	22	16	12	4.2	0		24	18	14	6.0	0		120	77	0.20	375	.	.
16	32	30	32	.5	1	22	14	12	3.7	0		22	16	14	4.9	0		560	69	0.13	.	.	.
19	28	30	34	1	1	22	16	12	4.2	0		20	14	12	3.4	0		175	70	0.25	.	.	.
22	32	34	36	.5	1	20	12	10	2.4	0		22	16	14	4.9	0		609	74	0.05	.	.	.
25	30	30	32	.5	1	20	14	12	3.4	0		22	18	14	5.5	0		489	74	0.28	356	.	.
28	26	28	26	.5	1	24	16	12	4.6	0		24	18	14	6.0	0		428	71	0.35	.	.	.
31	26	26	28	1	1	22	16	12	4.2	0		22	18	16	6.3	0		318	73	0.95	.	.	.
34	28	30	32	.5	1	22	18	14	5.5	1	RGC	22	16	14	4.9	0		631	74	0.35	.	.	.
37	28	28	28	1	1	22	18	14	5.5	0		24	16	14	5.4	0		576	73	0.38	356	.	3.0
40	28	26	28	.5	1	22	16	14	4.9	0		22	18	14	5.5	0		164	93	.	.	.	.
43	34	38	36	.5	1	22	18	14	5.5	0		24	20	16	7.7	0		379	77	.	.	.	.
46	32	32	34	.5	1	22	18	14	5.5	0		22	16	14	4.9	0		280	77	.	.	.	.
49	28	28	30	.5	1	20	16	14	4.5	0		24	20	16	7.7	0		142	69	.	370	.	.
52	26	26	28	1	1	22	20	16	7.0	0		24	18	16	6.9	0		368	69	.	.	.	.
55	26	24	26	1	1	24	20	16	7.7	0		22	16	14	4.9	0		159	80	.	.	.	.
58	26	26	26	1	1	22	20	16	7.0	1	MGC	22	16	14	4.9	0		208	69	.	.	.	.
61	26	26	26	1	1	24	22	18	9.5	1	LL	22	16	14	4.9	0		302	76	.	384	.	.
64	28	30	32	1	2	24	20	18	8.6	1	LGC	20	16	12	3.8	0		241	72	.	.	.	.
67	26	28	26	1	2	24	22	18	9.5	1	MGC	22	16	14	4.9	1	LM	247	74	.	.	.	.
70	28	30	28	1	1	16	12	10	1.9	0		22	18	14	5.5	0		115	76	.	.	.	.
73	26	26	28	1	1	26	22	20	11.4	1	LGC	22	18	14	5.5	0		307	76	.	388	.	.
76	24	26	26	1	1	22	20	18	7.9	1	LM	24	18	14	6.0	0		66	72	.	.	.	.
79	30	32	30	1	2	26	20	18	9.4	1	MGC	24	18	14	6.0	1	MGC	214	79	.	.	.	.
82	28	28	28	1	2	28	24	22	14.8	1	LM	22	18	14	5.5	1	TM	126	74	.	.	.	.
85	24	26	26	1	2	22	20	16	7.0	2	LM	22	16	12	4.2	0		65	81	.	397	.	.
88	26	26	26	1	2	26	20	18	9.4	2	LGC	22	16	14	4.9	0		6785	80	.	.	.	.
91	26	26	28	1	2	26	22	18	10.3	2	LGC	22	16	14	4.9	0		7581	79	.	411	127	4.3

TABLE A-17 CONTINUED POSTPARTUM DATA

COW NUMBER=23 TREATMENT=12 BREED=3

DAY	CVX	LHO	RHO	UTL	TON	LOL	LOH	LOW	LEFT OVARY	LST	LSL	ROL	ROH	ROW	RIGHT OVARY	RST	RSL	PROGESTERONE	GLUCOSE	LH	COW WT	CF WT	CON SCORE
1	.	.	.	.	.	.	.	.	.	.		.	.	.	.	.		394	95	0.25	318	32	6.3
4	.	.	.	.	.	.	.	.	.	.		.	.	.	.	.		572	75	0.23	.	.	.
7	.	.	.	.	.	.	.	.	.	.		.	.	.	.	.		164	85	.	.	.	.
10	40	48	50	.0	1	26	20	16	8.3	0		24	18	14	6.0	0		592	92	0.28	.	.	.
13	56	62	66	.0	1	24	18	14	6.0	0		28	20	18	10.1	0		266	87	0.35	300	.	.
16	34	38	36	.5	1	22	18	16	6.3	0		26	22	18	10.3	0		1219	75	0.28	.	.	.
19	28	28	30	.0	1	22	14	12	3.7	0		26	22	18	10.3	0		683	88	0.43	.	.	.
22	26	28	24	.0	1	22	22	18	8.7	1	MGC	24	20	16	7.7	0		1561	77	0.38	.	.	.
25	30	32	30	.5	1	24	20	14	6.7	1	TM	26	20	16	8.3	0		638	.	.	313	.	.
28	36	26	28	.5	1	22	22	18	8.7	0		24	20	18	8.6	0		522	60	0.28	.	.	.
31	24	24	26	.5	1	26	24	20	12.5	1	LM	28	24	20	13.4	1	MGC	966	83	0.53	.	.	.
34	26	26	28	.5	1	26	22	18	10.3	0		24	22	20	10.6	1	MGC	783	86	0.43	.	.	.
37	26	24	26	1	1	24	20	16	7.7	1	LM	26	22	18	10.3	1	MGC	561	87	0.48	316	.	4.3
40	28	28	26	.5	1	24	20	18	8.6	1	LM	26	22	20	11.4	0		300	85	.	.	.	.
43	28	30	28	1	1	24	20	18	8.6	1	LM	26	22	20	11.4	1	RGC	1483	80	.	.	.	.
46	24	26	24	1	1	22	18	16	6.3	1	LM	26	22	20	11.4	1	TM	905	86	.	.	.	.
49	26	28	26	.5	1	22	20	18	7.9	1	TM	26	22	20	11.4	1	RGC	1310	87	.	331	.	.
52	28	26	28	1	1	22	20	16	7.0	0		24	20	18	8.6	1	MGC	738	80	.	.	.	.
55	26	28	26	1	1	22	24	18	9.5	1	TM	24	22	20	10.6	1	TM	700	88	.	.	.	.
58	26	26	26	1	1	24	22	18	9.5	1	TM	24	20	18	8.6	1	LM	327	78	.	.	.	.
61	24	24	26	1	1	22	20	16	7.0	1	TM	24	20	16	7.7	1	TM	222	82	.	331	.	.
64	26	24	26	1	1	24	20	16	7.7	1	MGC	24	20	16	7.7	1	LR	650	74	.	.	.	.
67	28	28	30	1	2	24	22	18	9.5	1	LL	26	24	20	12.5	1	TM	164	78	.	.	.	.
70	26	28	28	1	1	22	20	16	7.0	1	TM	24	20	18	8.6	1	TM	1344	80	.	.	.	.
73	28	30	28	.5	1	24	18	14	6.0	1	TM	24	18	16	6.9	1	TM	388	80	.	347	.	.
76	28	28	30	1	2	26	20	18	9.4	1	LR	26	24	20	12.5	1	TM	422	75	.	.	.	.
79	24	26	26	1	2	22	20	16	7.0	0		28	24	22	14.8	2	MGC	133	72	.	.	.	.
82	26	26	26	1	2	24	20	16	7.7	1	TM	28	26	22	16.0	2	MGC	4849	75	.	.	.	.
85	26	26	26	1	2	22	18	14	5.5	0		30	28	24	20.2	2	MGC	5610	74	.	368	.	.
88	26	26	28	1	2	24	20	16	7.7	0		32	28	24	21.5	2	MGC	10700	77	.	359	86	6.0

TABLE A-17 CONTINUED POSTPARTUM DATA

COW NUMBER=24 TREATMENT=22 BREED=3

DAY	CVX	LHO	RHO	UTL	TON	LOL	LOH	LOW	LEFT OVARY	LST	LSL	ROL	ROH	ROW	RIGHT OVARY	RST	RSL	PRO GESTE RCNE	GLU CO SE	LH	COW WT	CF WT	CON SCO RE
1	.	.	.	.	.	.	.	.	.	.		.	.	.	.	.		710	87	0.00	406	45	7.0
3	.	.	.	.	.	.	.	.	.	.		.	.	.	.	.		915	74	0.00	.	.	.
6	.	.	.	.	.	.	.	.	.	.		.	.	.	.	.		1699	99	0.00	.	.	.
9	48	42	46	.0	1	24	16	14	5.4	0		22	18	14	5.5	0		1310	97	0.00	377	.	.
12	44	42	40	.0	1	20	16	14	4.5	0		20	12	10	2.4	0		1757	84	0.00	.	.	.
15	34	34	32	.5	1	22	16	14	4.9	0		26	16	14	5.8	0		1310	81	0.00	.	.	.
18	32	32	30	1	1	20	16	12	3.8	0		22	16	14	4.9	0		668	79	0.00	.	.	.
21	38	40	36	.5	1	20	16	12	3.8	0		26	18	14	6.6	0		1210	87	0.15	356	.	.
24	28	30	38	1	1	24	14	12	4.0	0		24	12	10	2.9	0		1399	83	0.13	.	.	.
27	26	30	38	.0	1	22	14	12	3.7	0		22	16	14	4.9	0		1457	82	0.23	.	.	.
30	28	26	28	1	1	22	14	12	3.7	0		24	14	12	4.0	0		4036	99	0.23	.	.	.
33	28	30	28	.5	1	22	14	12	3.7	0		22	12	10	2.6	0		1510	79	0.20	359	.	.
36	24	26	28	1	1	22	16	14	4.9	0		24	14	12	4.0	0		2563	73	0.15	.	.	.
39	24	28	26	1	1	20	14	10	2.8	0		24	14	12	4.0	0		484	72	0.28	.	.	5.0
42	24	26	24	.5	1	22	14	12	3.7	0		24	18	14	6.0	0		326	101	.	.	.	.
45	28	32	30	.5	1	24	16	14	5.4	0		22	16	12	4.2	0		2293	71	.	363	.	.
48	26	26	26	1	2	22	20	18	7.9	2	LGC	22	16	14	4.9	0		2231	72	.	.	.	.
51	26	28	26	1	1	24	18	14	6.0	0		22	16	12	4.2	0		1310	68	.	.	.	.
54	26	26	28	1	1	20	14	12	3.4	0		22	18	16	6.3	0		657	72	.	.	.	.
57	26	26	26	1	1	24	18	14	6.0	0		22	16	14	4.9	0		352	77	.	377	.	.
60	26	26	26	.5	1	20	14	12	3.4	0		22	16	14	4.9	0		805	66	.	.	.	.
63	26	28	28	1	1	22	16	14	4.9	0		22	18	14	5.5	0		963	71	.	.	.	.
66	28	26	26	1	1	24	18	14	6.0	0		24	20	16	7.7	0		431	62	.	.	.	.
69	24	26	26	1	1	22	16	14	4.9	0		24	18	14	6.0	0		736	57	.	388	.	.
72	26	26	28	1	1	22	18	14	5.5	0		20	14	12	3.4	0		768	65	.	.	.	.
75	26	26	26	1	1	22	16	14	4.9	0		20	14	12	3.4	0		647	64	.	.	.	.
78	24	24	26	1	1	22	18	14	5.5	1	LL	20	14	12	3.4	0		736	60	.	.	.	.
81	24	24	26	1	1	20	18	14	5.0	0		20	14	12	3.4	1	MGC	410	62	.	406	.	.
84	28	28	28	1	1	22	16	14	4.9	1	MGC	22	16	14	4.9	0		847	58	.	.	.	.
87	26	24	26	1	1	24	20	16	7.7	1	RGC	24	20	16	7.7	1	TL	347	56	.	.	.	.
90	26	28	26	1	1	22	18	14	5.5	1	TM	22	16	14	4.9	0		468	66	.	397	107	6.3

TABLE A-17 CONTINUED POSTPARTUM DATA

COW NUMBER=25 TREATMENT=11 BREED=2

DAY	CVX	LHO	RHO	UTL	TON	LOL	LOH	LOW	LEFT OVARY	LST	LSL	ROL	ROH	ROW	RIGHT OVARY	RST	RSL	PROGESTERONE	GLUCOSE	LH	COW WT	CF WT	CON SCORE
0	.	.	.	.	.	.	.	.	.	.		.	.	.	.	.		472	92	0.00	336	32	7.0
4	.	.	.	.	.	.	.	.	.	.		.	.	.	.	.		451	62	0.00	.	.	.
7	.	.	.	.	.	.	.	.	.	.		.	.	.	.	.		279	71	0.00	.	.	.
10	54	46	44	.0	1	26	20	16	8.3	0		32	22	18	12.7	0		912	60	0.00	340	.	.
13	38	34	36	.0	1	22	18	16	6.3	0		26	20	16	8.3	0		1380	79	0.13	.	.	.
16	38	36	36	1	1	26	18	14	6.6	0		26	20	16	8.3	0		547	63	0.05	.	.	.
19	34	34	36	.5	1	24	22	18	9.5	0		28	24	20	13.4	0		456	75	0.00	.	.	.
22	30	30	28	1	1	22	18	14	5.5	0		24	16	14	5.4	0		231	78	0.00	338	.	.
25	26	26	28	1	1	24	20	16	7.7	0		26	22	18	10.3	0		1519	70	0.00	.	.	.
28	30	34	32	1	1	22	16	14	4.9	0		24	20	16	7.7	0		338	70	0.00	.	.	.
31	26	24	26	1	1	24	18	16	6.9	1	TM	26	18	14	6.6	0		601	80	0.00	.	.	.
34	24	28	26	1	1	22	14	12	3.7	0		28	22	18	11.1	0		923	78	0.13	338	.	.
37	26	28	30	1	1	22	16	14	4.9	0		26	22	20	11.4	0		864	66	0.05	.	.	5.0
40	28	30	34	1	1	22	16	12	4.2	0		24	20	16	7.7	0		91	87	.	.	.	.
43	26	24	24	1	1	22	16	14	4.9	0		24	20	18	8.6	0		2604	80	.	.	.	.
46	30	30	32	1	1	22	16	14	4.9	0		26	22	18	10.3	0		435	64	.	327	.	.
49	24	28	26	1	1	24	18	14	6.0	0		24	20	16	7.7	0		660	66	.	.	.	.
52	24	28	26	1	1	24	18	16	6.9	0		28	22	18	11.1	0		644	76	.	.	.	.
55	28	28	30	1	1	24	20	16	7.7	1	LM	26	22	18	10.3	0		601	61	.	.	.	.
58	24	24	26	1	1	22	18	14	5.5	0		24	20	16	7.7	0		537	67	.	329	.	.
61	24	24	26	1	1	22	16	14	4.9	1	LM	24	18	14	6.0	0		494	68	.	.	.	.
64	28	30	28	1	1	26	22	18	10.3	0		26	22	18	10.3	0		386	72	.	.	.	.
67	24	26	24	1	1	22	18	14	5.5	0		24	20	16	7.7	0		85	69	.	.	.	.
70	26	26	28	1	1	20	16	14	4.5	0		26	20	18	9.4	0		322	67	.	352	.	.
73	28	28	28	1	1	20	16	14	4.5	0		26	22	20	11.4	1	LM	257	72	.	.	.	.
76	26	26	28	1	1	22	18	14	5.5	1	TM	24	22	18	9.5	0		402	71	.	.	.	.
79	26	26	26	1	1	24	18	16	6.9	1	TM	26	22	20	11.4	1	LR	290	70	.	.	.	.
82	28	30	28	1	1	24	18	16	6.9	1	LL	24	20	16	7.7	1	LM	209	65	.	356	.	.
85	26	28	28	1	2	24	20	16	7.7	0		26	22	20	11.4	0		257	71	.	.	.	.
88	26	26	26	1	2	26	22	20	11.4	2	LGC	26	20	18	9.4	1	LGC	1680	75	.	.	.	.
91	26	26	28	1	2	26	22	18	10.3	2	LGC	24	20	16	7.7	0		703	67	.	338	95	7.0

TABLE A-17 CONTINUED POSTPARTUM DATA

COW NUMBER=26 TREATMENT=21 BREED=3

DAY	CVX	LHO	RHO	UTL	TON	LOL	LOH	LOW	LEFT OVARY	LST	LSL	ROL	ROH	ROW	RIGHT OVARY	RST	RSL	PROGESTERONE	GLUCOSE	LH	COW WT	CF WT	CON SCORE
0	.	.	.	.	.	.	.	.	.	.		.	.	.	.	.		581	69	0.00	397	41	6.7
2	.	.	.	.	.	.	.	.	.	.		.	.	.	.	.		262	63	0.00	.	.	.
5	.	.	.	.	.	.	.	.	.	.		.	.	.	.	.		306	76	0.00	.	.	.
8	.	.	.	.	.	.	.	.	.	.		.	.	.	.	.		356	60	0.00	.	.	.
11	42	45	50	.0	1	34	26	20	17.7	0		30	20	16	9.6	0		141	66	0.18	.	.	.
14	50	40	48	.0	1	26	20	16	8.3	0		34	22	18	13.5	0		356	64	0.10	402	.	.
17	45	40	38	.0	1	22	18	14	5.5	0		24	20	16	7.7	0		506	68	0.00	.	.	.
20	36	34	36	.0	1	26	22	18	10.3	0		24	20	16	7.7	0		237	67	0.00	.	.	.
23	34	34	36	.0	1	28	20	18	10.1	0		32	24	18	13.8	0		268	64	0.00	.	.	.
26	26	26	28	.5	1	32	22	18	12.7	0		24	18	16	6.9	0		268	66	0.00	411	.	.
29	30	30	34	.5	1	22	20	16	7.0	0		26	22	18	10.3	0		1468	67	0.20	.	.	.
32	26	24	26	1	1	26	22	20	11.4	0		24	20	18	8.6	0		549	63	0.00	.	.	.
35	28	28	30	.5	1	26	20	16	8.3	0		26	24	20	12.5	0		285	64	0.00	.	.	.
38	30	30	32	1	1	22	16	14	4.9	0		26	22	18	10.3	0		462	66	0.10	379	.	5.0
41	.	.	.	.	.	.	.	.	.	.		.	.	.	.	.		268	.	.	.	.	.
44	32	32	34	.5	1	24	20	14	6.7	0		22	20	16	7.0	0		212	63	.	.	.	.
47	28	36	36	.5	1	26	20	14	7.3	0		24	20	16	7.7	0		292	67	.	.	.	.
50	26	30	28	.5	1	24	20	16	7.7	0		26	22	18	10.3	0		277	64	.	397	.	.
53	26	26	28	.5	1	22	18	14	5.5	0		26	22	18	10.3	0		274	61	.	.	.	.
56	24	26	24	1	2	22	16	14	4.9	0		24	18	16	6.9	0		581	59	.	.	.	.
59	26	28	30	1	1	26	22	18	10.3	0		26	22	20	11.4	0		443	56	.	.	.	.
62	26	28	26	1	1	24	20	18	8.6	1	LM	24	20	18	8.6	0		610	67	.	427	.	.
65	26	28	30	.5	1	24	20	16	7.7	0		26	22	18	10.3	0		405	68	.	.	.	.
68	30	32	30	.5	1	26	22	20	11.4	1	TM	26	20	18	9.4	0		159	69	.	.	.	.
71	26	28	28	1	1	24	20	16	7.7	0		24	20	18	8.6	0		262	69	.	.	.	.
74	26	26	28	1	1	24	20	18	8.6	1	MGC	26	22	20	11.4	0		1068	68	.	429	.	.
77	28	30	28	1	1	32	28	24	21.5	1	LL	26	20	16	8.3	0		1587	66	.	.	.	.
80	30	34	32	.5	1	24	20	18	8.6	1	TM	28	24	22	14.8	1	MGC	600	66	.	.	.	.
83	26	28	28	1	1	26	24	22	13.7	2	LGC	24	20	18	8.6	1	LL	2818	69	.	.	.	.
86	28	30	28	.5	2	32	28	26	23.3	2	MGC	28	22	18	11.1	0		5368	59	.	422	.	.
89	26	24	26	1	2	34	26	24	21.2	2	MGC	24	20	16	7.7	0		11152	54	.	413	100	6.0

TABLE A-17 CONTINUED POSTPARTUM DATA

COW NUMBER=27 TREATMENT=21 BREED=4

DAY	CVX	LHO	RHC	UTL	TON	LOL	LOH	LOW	LEFT OVARY	LST	LSL	ROL	ROH	ROW	RIGHT OVARY	RST	RSL	PROGESTERONE	GLUCOSE	LH	COW WT	CF WT	CON SCORE
1	.	.	.	.	.	.	.	.	.	.		.	.	.	.	.		626	68	0.00	411	48	6.3
5	.	.	.	.	.	.	.	.	.	.		.	.	.	.	.		362	56	0.00	.	.	.
8	.	.	.	.	.	.	.	.	.	.		.	.	.	.	.		614	64	0.15	.	.	.
11	50	44	46	.0	1	24	18	16	6.9	0		22	18	16	6.3	0		362	49	0.18	427	.	.
14	36	36	38	.0	1	20	18	14	5.0	0		20	16	12	3.8	0		310	56	0.13	.	.	.
17	38	42	40	.5	1	22	18	14	5.5	0		20	16	12	3.8	0		442	58	0.05	.	.	.
20	36	40	38	.5	1	22	18	16	6.3	0		20	16	14	4.5	0		419	56	0.00	.	.	.
23	36	38	36	.5	1	22	16	12	4.2	0		20	16	14	4.5	0		614	57	0.00	418	.	.
26	32	36	32	.5	1	24	20	16	7.7	0		26	22	18	10.3	0		534	62	0.25	.	.	.
29	24	26	24	.5	1	22	18	14	5.5	0		24	20	18	8.6	0		356	55	0.20	.	.	.
32	30	32	34	.5	1	22	20	14	6.2	0		22	18	14	5.5	0		523	79	0.23	.	.	.
35	34	40	36	.5	1	20	16	12	3.8	0		18	16	14	4.0	0		517	58	0.05	409	.	.
38	36	44	40	.0	1	20	14	12	3.4	0		24	20	16	7.7	0		471	52	0.25	.	.	5.3
41	30	34	28	1	1	20	14	10	2.8	0		22	16	12	4.2	0		356	60	.	.	.	.
44	32	38	34	.5	1	24	16	14	5.4	0		20	16	12	3.8	0		0	57	.	.	.	.
47	32	34	32	.5	1	22	16	12	4.2	0		20	14	12	3.4	0		1017	66	.	400	.	.
50	30	34	32	.5	1	20	18	16	5.8	0		22	16	14	4.9	0		517	62	.	.	.	.
53	34	38	36	.5	1	22	16	14	4.9	0		22	18	14	5.5	0		161	53	.	.	.	.
56	26	28	26	.5	1	22	18	14	5.5	0		24	18	16	6.9	0		1109	51	.	.	.	.
59	28	32	28	.5	1	26	20	18	9.4	1	TM	24	20	18	8.6	0		2390	56	.	418	.	.
62	32	30	32	1	2	22	18	14	5.5	0		22	18	16	6.3	0		362	72	.	.	.	.
65	28	30	28	1	1	24	18	14	6.0	0		22	20	18	7.9	0		1557	65	.	.	.	.
68	30	32	28	.5	1	24	18	14	6.0	0		28	22	20	12.3	1	TM	3695	62	.	.	.	.
71	28	30	28	1	1	24	20	16	7.7	1	RGC	26	22	20	11.4	1	TM	5568	60	.	436	.	.
74	26	26	28	1	2	22	18	16	6.3	1	RGC	24	22	20	10.6	2	LGC	5390	59	.	.	.	.
77	28	28	26	.5	2	24	18	16	6.9	1	RGC	26	22	20	11.4	2	LGC	7839	55	.	.	.	.
80	28	30	28	.5	2	20	16	14	4.5	0		24	22	20	10.6	2	LGC	5207	56	.	427	100	4.7

TABLE A-17 CONTINUED POSTPARTUM DATA

COW NUMBER=28 TREATMENT=11 BREED=5

DAY	CVX	LHO	RHO	UTL	TON	LOL	LOH	LOW	LEFT OVARY	LST	LSL	ROL	ROH	ROW	RIGHT OVARY	RST	RSL	PROGESTERONE	GLUCOSE	LH	COW WT	CF WT	CONSCORE
0	.	.	.	.	.	.	.	.	.	.		.	.	.	.	.		944	83	0.28	481	39	7.0
3	.	.	.	.	.	.	.	.	.	.		.	.	.	.	.		333	79	0.33	.	.	.
6	.	.	.	.	.	.	.	.	.	.		.	.	.	.	.		405	66	0.23	.	.	.
9	40	58	54	.0	1	22	14	12	3.7	0		22	18	14	5.5	0		305	67	0.45	.	.	.
12	36	44	48	.0	1	26	16	12	5.0	0		30	16	14	6.7	0		355	69	0.38	484	.	.
15	36	36	38	.0	1	22	14	12	3.7	0		22	18	14	5.5	0		288	67	0.50	.	.	.
18	46	48	48	.0	1	22	14	12	3.7	0		26	16	14	5.8	0		611	67	0.45	.	.	.
21	38	36	38	.5	1	22	18	14	5.5	0		22	16	14	4.9	0		366	67	0.48	.	.	.
24	34	36	34	.5	1	24	18	16	6.9	1	RGC	26	18	14	6.6	0		366	66	0.65	486	.	.
27	30	32	36	1	1	24	16	14	5.4	1	MGC	24	16	14	5.4	0		305	74	0.80	.	.	.
30	34	32	32	.5	1	24	14	12	4.0	0		24	16	14	5.4	0		588	68	0.75	.	.	.
33	30	32	32	.5	1	24	20	18	8.6	1	LR	22	16	14	4.9	0		2866	63	0.48	.	.	.
36	32	34	32	.5	1	20	18	14	5.0	0		22	14	12	3.7	0		694	64	.	486	.	.
39	32	34	32	.5	1	24	20	16	7.7	0		22	14	12	3.7	0		433	63	0.53	.	.	5.7
42	32	30	32	.5	1	24	20	16	7.7	1	MGC	24	16	14	5.4	0		2677	64	.	.	.	.
45	28	28	26	.5	1	24	20	18	8.6	1	MGC	22	14	12	3.7	0		6982	67	.	.	.	.
48	26	26	26	1	1	24	20	16	7.7	1	MGC	20	16	14	4.5	0		4960	66	.	470	.	.
51	28	28	26	1	1	24	18	16	6.9	1	LM	20	16	14	4.5	0		6749	81	.	.	.	.
54	28	28	28	1	1	22	18	16	6.3	0		22	16	14	4.9	0		277	59	.	.	.	.
57	28	30	30	1	1	22	16	14	4.9	1	TM	22	16	14	4.9	0		477	75	.	.	.	.
60	28	26	26	1	2	22	16	12	4.2	1	TM	24	16	14	5.4	2	LGC	1766	72	.	490	.	.
63	28	30	28	1	2	20	14	12	3.4	0		26	20	18	9.4	2	LGC	4599	64	.	.	.	.
66	28	28	28	1	2	22	14	12	3.7	0		26	20	18	9.4	2	LGC	7010	72	.	.	.	.
69	28	28	28	1	2	22	14	12	3.7	0		26	20	16	8.3	2	LGC	8232	77	.	.	.	.
72	28	28	28	1	2	22	16	14	4.9	0		24	20	16	7.7	2	LGC	1922	69	.	499	.	.
75	28	28	30	1	2	20	12	10	2.4	0		26	22	20	11.4	2	MGC	9849	75	.	.	.	.
78	28	28	30	1	2	24	16	14	5.4	1	LR	26	20	18	9.4	2	LGC	799	69	.	.	.	.
81	30	32	30	1	1	22	16	12	4.2	1	RGC	22	18	14	5.5	1	RGC	399	67	.	.	.	.
84	28	30	30	1	1	22	16	14	4.9	1	TM	22	14	12	3.7	0		377	66	.	513	.	.
87	28	28	30	1	1	22	20	16	7.0	1	MGC	20	16	12	3.8	0		2214	71	.	.	.	.
90	26	26	28	1	2	26	20	16	8.3	2	MGC	20	14	12	3.4	0		4856	63	.	522	118	6.3

TABLE A-17 CONTINUED POSTPARTUM DATA

COW NUMBER=29 TREATMENT=21 BREED=5

DAY	CVX	LHO	RHO	UTL	TON	LOL	LOH	LOW	LEFT OVARY	LST	LSL	ROL	ROH	ROW	RIGHT OVARY	RST	RSL	PROGESTERONE	GLUCOSE	LH	COW WT	CF WT	CON SCORE
0	.	.	.	.	.	.	.	.	.	.		.	.	.	.	.		572	74	0.15	424	48	6.3
2	.	.	.	.	.	.	.	.	.	.		.	.	.	.	.		700	79	0.25	.	.	.
5	.	.	.	.	.	.	.	.	.	.		.	.	.	.	.		1288	87	0.15	.	.	.
8	.	.	.	.	.	.	.	.	.	.		.	.	.	.	.		850	73	0.13	.	.	.
11	44	46	48	.0	1	26	18	16	7.5	0		26	22	20	11.4	0		1863	76	0.30	400	.	.
14	40	48	46	.0	1	22	16	14	4.9	0		26	22	16	9.2	0		4249	95	0.05	.	.	.
17	42	40	42	.5	1	24	18	14	6.0	0		24	18	14	6.0	0		3033	96	0.05	.	.	.
20	34	34	36	.0	1	26	16	14	5.8	0		24	18	14	6.0	0		1744	83	.	.	.	.
23	36	42	40	.0	1	26	18	14	6.6	0		26	18	14	6.6	0		2227	83	0.20	390	.	.
26	32	34	36	.0	1	24	18	14	6.0	0		22	16	12	4.2	0		1394	82	0.25	.	.	.
29	32	36	34	.5	1	24	16	12	4.6	0		26	16	14	5.8	0		1694	90	0.20	.	.	.
32	28	30	28	.5	1	30	22	20	13.2	1	TL	24	20	18	8.6	0		2733	73	0.20	.	.	.
35	32	32	30	.5	1	26	24	22	13.7	1	MGC	28	20	18	10.1	0		4188	75	0.18	393	.	.
38	32	36	34	.5	1	22	18	16	6.3	0		24	20	18	8.6	0		1866	79	0.35	.	.	.
41	28	26	30	.5	1	24	18	16	6.9	0		22	18	12	4.8	0		2455	79	0.25	.	.	5.0
44	32	36	32	.5	1	20	14	12	3.4	0		24	16	14	5.4	0		3277	87	.	.	.	.
47	28	28	30	.5	1	24	16	14	5.4	0		26	20	18	9.4	0		10123	83	.	372	.	.
50	28	28	28	1	1	22	16	12	4.2	0		22	20	18	7.9	0		12291	82	.	.	.	.
53	32	32	30	.5	1	24	18	14	6.0	0		24	20	16	7.7	0		2860	83	.	.	.	.
56	30	32	34	.5	1	22	16	12	4.2	0		24	20	16	7.7	0		955	102	.	.	.	.
59	26	28	26	1	1	24	20	16	7.7	0		26	20	16	8.3	1	LL	833	86	.	393	.	.
62	26	26	24	1	2	20	16	14	4.5	0		26	20	16	8.3	2	MGC	972	72	.	.	.	.
65	28	26	28	1	2	22	16	14	4.9	0		24	22	18	9.5	2	LGC	2349	65	.	.	.	.
68	28	28	28	1	2	24	14	12	4.0	0		24	18	14	6.0	2	LGC	1338	78	.	.	.	.
71	26	26	28	1	2	24	16	14	5.4	0		24	20	16	7.7	2	LGC	283	79	.	397	.	.
74	28	28	30	1	2	22	18	14	5.5	0		22	16	14	4.9	1	LR	461	64	.	.	.	.
77	28	30	30	1	1	22	16	12	4.2	0		24	18	16	6.9	1	MGC	438	73	.	.	.	.
80	28	28	30	1	2	22	16	12	4.2	0		22	18	16	6.3	1	LM	544	72	.	.	.	.
83	24	26	26	1	1	22	16	14	4.9	0		22	18	14	5.5	1	LR	988	83	.	411	.	.
86	26	28	28	1	1	22	14	12	3.7	0		24	18	14	6.0	1	LM	2494	66	.	.	.	.
89	30	32	30	.5	1	22	16	14	4.9	1	LL	22	16	14	4.9	1	TM	477	70	.	429	129	5.3

Abbreviations used in the table A-17

Treatment - 11 = cows fed monensin and injected with E_2
12 = cows fed monensin and injected with GnRH
21 = cows fed control supplement and injected with E_2
22 = cows fed control supplement and injected with GnRH
Breed - 1 = Hereford x Angus, 2 = Angus x Hereford, 3 = Penzgauer x Angus, 4 = Penzgauer x Hereford, 5 = Brown Swiss x Angus
Day = day postpartum
CVX = cervix diameter (mm)
LHO = left horn diameter (mm)
RHO = right horn diameter (mm)
UTL = uterine location (0 = abdominal cavity, .5 = half way between abdominal and pelvic cavities, 1 = pelvic cavity)
TON = tonus of the uterus (1 = flacid without any tonus, 2 = tonus typical of a cycling cow)
LOL = left ovarian length (mm)
LOH = left ovarian height (mm)
LOW = left ovarian width (mm)
LEFT OVARY = left ovarian volume (cm^3)
LST = left ovarian structure type (0 = no structure, 1 = follicle greater than 6 mm, 2 = corpus luteum)
LSL = left ovarian structure location (TR = top right, TM = top middle, TL = top left, RGC = right greater curvature, MGC = middle greater curvature, LGC = left greater curvature, LR = lower right, LM = lower middle, LF = lower left)
ROL = right ovarian length (mm)
ROH = right ovarian height (mm)
ROW = right ovarian width (mm)
RIGHT OVARY = right ovarian volume (cm^3)
RST = right ovarian structure type
RSL = right ovarian structure location
PROGESTERONE = progesterone concentration (pg/ml)
GLUCOSE = glucose (mg/dl)
LH = luteinizing hormone (ng/ml)
COW WT = cow weight (kg)
CALF WT = calf weight (kg)
CON SCORE = condition score (the first condition score in the table is the one assigned about 65 days before parturition)

TABLE A-18. DAYS FROM PARTURITION TO FIRST ESTRUS AND TO HORMONE CHALLENGE WITH E_2 OR GnRH

Cow number	First estrus	E_2[a]	GnRH[a]
01		39	
02			43
03	73	42	
05	78		42
06			41
07		45	
08	59		40
09	89	41	
10	56		42
11	38		41
12	59		42
13	87		41
14	66		40
15			39
16	66		42
17			43
18	78	42	
19		44	
20	69	40	
21	33		42
22	81	40	
23	75		39
24			41
25	84	40	
26	79	41	
27	60	39	
28	36	40	
29	38	44	

[a] E_2 = estradiol-17β (10 mg, i.m.), GnRH = gonadotropin releasing hormone (100 ug, i.v.)

Laboratory Analysis

Reagents for LH Radioimmunoassay

1. Sodium phosphate monobasic ($NaH_2PO_4 \cdot H_2O$) 0.5 M.
    a. $NaH_2PO_4 \cdot H_2O$ - 69 g.
    b. deionized water (dH_2O).
    c. Add NaH_2PO_4 (69 g) to 1000 ml volumetric flask and add 500 ml of dH_2O. Place on magnetic stirrer until completely dissolved. Remove magnet. Bring to 1000 ml volume. Store at 0 to 4° C.
2. Sodium phosphate dibasic (Na_2HPO_4) 0.5 M.
    a. Na_2HPO_4 - 70.98 g.
    b. dH_2O.
    c. Add Na_2HPO_4 (70.98 g) to 1000 ml volumetric flask. Add 500 ml luke warm dH_2O. Place on magnetic stirrer. When dissolved bring to room temperature and bring to 1000 ml volume. Store at 0 to 4° C.
3. Phosphate buffer system plus merthiolate (PBS-MER) pH 7.0.
    a. to prepare 3500 ml:

NaCl	28.6 g
Merthiolate (Thimerosal)	0.35 g
NaH_2PO_4 0.5 M	48 ml
Na_2HPO_4 0.5 M	24 ml
dH_2O up to	3500 ml

    b. Weigh NaCl and transfer to 1000 ml beaker. Add 500 ml of dH_2O. Add merthiolate and dissolve. Add the two phosphate solutions. Transfer to 1000 ml graduated cylinder. Make up volume to 1000 ml. Transfer to storage bottle and make up to 3500 ml with dH_2O washing beaker and cylinder several times.

4. Phosphate buffer (PB) 0.5 M pH 7.4.
    a. Mix the 0.5 M dibasic and monobasic sodium phosphate solutions to give a pH 7.4. (± 84 ml of Na_2HPO_4 solution and 16 ml NaH_2PO_4 solution - add each one until pH is corrected).
    b. Store frozen some PB in 1 ml aliquots for LH iodination and keep the other in larger volumes of 200-300 ml and frozen to avoid bacterial growth.
5. Phosphate buffer and Merthiolate (PB-MER) 0.05 M pH 7.4.
    a. Dilute the 0.5 M PB pH 7.4 1:10 to give 0.05 M pH 7.4.
    b. Add 100 mg of Merthiolate to 1000 ml of 0.05 M PB pH 7.4.
6. One (1) % Bovine serum albumin (BSA) PB-MER 0.05 M pH 7.4 (1% BSA).
    a. Put 10 g of BSA in a 1000 ml volumetric flask and complete to volume with PB-MER 0.05 M pH 7.4.
7. Ethylene dinitrilo tetracetic acid, disodium salt (EDTA) pH 7.0.
    a. EDTA 18.6 g.
    b. PBS-MER pH 7.0 1000 ml.
    c. Warm up to dissolve in about 200 ml of PBS-MER pH 7.0. Add 700 ml of PBS-MER pH 7.0. Adjust pH to 7.0 with 5 N NaOH. Complete volume to 1000 ml.

Reagents for LH Iodination

1. Chloramine-T 0.5 ug/ul.
    a. Dilute 2 mg of chloramine-T with 4 ml of PB 0.25 M pH 7.4. Protect the solution from strong light. Make fresh at the time of use.
2. Phosphate buffer 0.25 M pH 7.4.
    a. Dilute PB 0.5 M pH 7.4 to 1:2 with dH_2O.
3. Sodium metabisulfite ($Na_2S_2O_5$) 5 ug/ul.
    a. Dilute 5 mg of $Na_2S_2O_5$ with 1 ml of PB 0.25 M pH 6.7. Make fresh on day of use.
4. Phosphate buffer 0.25 M pH 6.7.
    a. Mix the 0.5 M dibasic and monobasic phosphate solutions to give pH 6.7 (± 56.5 ml of monobasic and ± 43.5 ml of dibasic phosphate - add each one until pH is correct). Dilute the solution 1:1 to give 0.25 M pH 6.7.
5. Transfer solution.
    a. Add the following reagents to a 10 ml volumetric flask:
        100.00 mg KI
        1.0 mg bromphenol blue
        1.6 g sucrose
    b. Dilute to mark with dH_2O and store frozen in .5 ml aliquots.
6. Rinse solution.
    a. Add the following reagents to a 10 ml volumetric flask:
        100.00 mg KI
        1.0 mg bromphenol blue
        0.8 g sucrose

   b. Dilute to mark with dH_2O and store frozen in .5 ml aliquots.

7. Egg albumin (EA)-PBS-MER-5%.

   a. Egg albumin 50 g

      PBS-MER 1 l

   b. Add 500 ml of PBS-MER, put it over magnetic stirrer and add EA little by little. Stir for two hours. Centrifuge (7,000 RPM for 20 minutes) and remove supernatant. Make up supernatant volume of 1 l with PBS-MER. Adjust pH to 7.4 with diluted NaOH. Freeze in 50 ml aliquots (22 ml are needed for each iodination).

Preparation of Disposable Columns of Bio-Gel P-60

1. Slowly add dry gel particles to 0.05 M PB-MER pH 7.4 with agitation. Allow to stand overnight at 4° C. Store indefinitely at 4° C.
2. Bring slurry to room temperature. Place in vacuum desiccator and evacuate for at least 20 min.
3. Place glass bead in the bottom of a disposable column, 1 x 20 cm. Place a pen mark 12 cm above the glass bead. Stopper bottom with rubber tubing and stopcock.
4. With small beaker underneath, rinse column with 0.05 M PB-MER pH 7.4 and then fill column 1/3 of the way with the same buffer. Add well mixed slurry. When settling is well underway, open outlet and allow to run freely. Continue to add slurry as needed. Never allow the top to settle before adding more. When meniscus reaches mark run only PB-MER through.
5. Cut a filter paper (e.g. Whatman #541) in a small circle that exactly fits in the column and then settle it on top of the gel.
6. Add 1.5 ml of 5% EA-PBS-MER and allow to run through the column.
7. Rinse column with 0.05 PB-MER pH 7.4 for 10 minutes, having about .1 cm of buffer on top of the gel (ready to use).
8. If storae of the column for 24 h is desirable leave about 2 cm of buffer on top of the gel and keep it at room temperature. If longer, store at 4° C for up to 2 weeks. Outlet should be closed and top covered with parafilm.

Procedure for Iodination

1. Take one vial containing 5 ug of lyophilized LH from the freezer.
2. Add 30 ul of PB 0.5 M pH 7.4. Mix.
3. Add 1 mCi I^{125} in 10 ul. Mix.
4. Add 10 ul of freshly prepared chloramine-T. (5 ug/10 ul). Mix gently by tapping and rotating the vial for exactly 2 minutes.
5. Add Na metabisulfate (250 ug/50 ul). Mix for 15 seconds.
6. Add 100 ul of transfer solution.
7. Layer on the surface of a Bio-Gel P-60 column with pasteur pipette (just above filter paper). Avoid contact with column wall.
8. Open stopcock. Start counting drops.
9. Add 50 ul rinse solution to vial and transfer to column.
10. Wash down sides of column with PB 0.05 M pH 7.4.
11. Collect 10 drops per tube from tube number 1 to 20, to which 1.0 ml of 5% EA-PBS-MER has been added previously.
12. Vortex tubes gently. Transfer 25 ul to 12 x 75 mm culture tubes.
13. Count for .5 minute each.
14. Use the first peak tube (usually number 5) or the tube immediately after the peak if more iodinated LH is needed.
15. Store at 4° C in concentrated form.
16. Dilute as needed, using 1% BSA (BSA-PB-MER-.05 M-pH 7.4).
17. Discard excess reagents, disposable pipettes, tubes etc. in the proper waste place.
18. Put data on iodination sheet.

Dilutions of LH

1. LH for radioiodination.

   USDA - bLH-I-1

   a. 248.5 micrograms (ug) were weighed and 4.97 ml of deionized H_2O added to give a dilution of 5.0 ug/100 ul.

   b. 100 ul was transferred to each vial, frozen, lyophilized and stored frozen.

   The vials, before use, were cleaned by boiling them in a 2% HCl solution, the caps were cleaned with alcohol, and both rinsed with dH_2O.

2. LH for standard.

   USDA-bLH-B-5

   a. 951 ug were weighed, 1 ml of dH_2O was added to dissolve, and then 94.1 ml of 1% BSA (BSA-PB-MER-.05 M-pH 7.4) were added. This is the STOCK-1 which has 10 ug/ml.

   b. 10 ml of STOCK-1 was transferred to a 2000 ml volumetric flask and brought to the volume with 1% BSA. This solution gave a 5 ng/100 ul.

   Four milliliters of the dilution 5 ng/100 ul were transferred to vials that were previously washed in 2% HCl solution (24 h bath at room temperature) and rinsed with dH_2O. The vials content was frozen, lyophilized and subsequently stored frozen. To reconstitute add 4 ml of dH_2O.

LH Radioimmunoassay

1. Preparing the samples (100 and 200 ul).

   Four hundred or 300 ul of 1% BSA were added to each (12 x 75 mm borosilicate tubes) and then the samples of 100 or 200 ul were added to complete a volume of 500 ul. Borosilicate tubes were used to obtain low non-specific binding. Sample volumes of up to 300 ul can be assayed.

2. Standard LH - (USDA-bLH-B-5).

   Vials containing 200 ng of LH plus buffer salts and BSA were reconstituted by adding 4 ml of dH_2O, resulting in 5 ng of LH/100 ul.

   From the 5 ng/100 ul solution, 100 ul were transferred to another vial and 900 ul of 1% BSA were added which gave a solution of 0.5 ng/100 ul.

   The solution was used for pipetting standards of .06, .12, .25 and .5 ng per tube (12 to 100 ul) of the standard curve while the solution 5 ng/100 ul was used for additions of 1, 2.5, 5 and 10 ng per tube (20 to 200 ul) of the standard curve.

3. Normal rabbit serum (NRS) dilution.

   NRS was diluted in EDTA-PBS to a ratio of 1:400.

4. First anti-body (1st AB) dilution (AB-B-225-Niswender).

   The 1st AB was stored in vials containing 100 ul of 1:400 dilution.

   The ratio of dilution of 1st AB depends on a titer check that goes from 1:60,000 to 1:200,000. Dilution used in the experiment was 1:140,000. 100 ul (content of one vial; 100 ul of 1:400) was defrosted and transferred to a beaker containing

34.9 ml of NRS 1:400.

For titer check dilution see table A-19.

5. Second anti-body (2nd AB) dilution.
    a. Sheep antirabbit guinea pig γ globulin serum (SARGPGG) was diluted in 1:400 NRS as indicated by the titer check that goes from 1:2 to 1:35. Dilution used in the experiment was 1:30 (1 ml of 2nd AB + 29 ml of NRS 1:400).
    b. Polyethylene glycol (PEG) 5% solution.
       PEG - 6,000 flake, M.W. - 6,000-7,500.
       PEG solution was made up in PB 0.05 M pH 7.4.
    c. The dilution ratio was 200 ml of 2nd AB solution (1:30) to 500 ml of PEG - 6%. Seven hundred microliters were used per tube in the assay.
    d. Titer checking of 1st AB and 2nd AB were done using 1% egg albumin (table A-20) and 1% bovine serum albumin (table A-21).
6. Iodinated LH (LH^{125}) dilution.

   LH^{125} was diluted in 1% BSA, at the time of use, to give 50,000 counts per minute (CPM)/100 ul.
7. For inter- and intraassay coefficients of variation utilize:
    a. Ovariectomized plasma (OVX). In triplicates of 100, 200 and 300 ul.
    b. OVX plus LH (2 ng/ml). Two nanogram of LH (USDA-bLH-B-5) were added to each ml of OVX and assayed in triplicates of 100, 200 and 300 ul.

TABLE A-19. FIRST ANTI-BODY DILUTION FOR TITER CHECK

Volume		NRS 1:400 (ml)	1st AB dilution
I	0.1 ml of 1:400*	14.9	1:60,000
II	1.5 ml of 1:60,000	0.5	1:80,000
III	1.5 ml of 1:60,000	1.0	1:100,000
IV	1.0 ml of 1:60,000	1.0 ml	1:120,000
V	1.0 ml of 1:60,000	1.33 ml	1:140,000

*1st AB was stored in vials containing 100 ul of 1:400 dilution.

Calculation for the dilution of 1:140,000:

$$\begin{array}{ll} 0.1\text{ ml} & 1:400 \\ x & 1:140{,}000 \end{array} \qquad x = \frac{140000}{400} = 35\text{ ml (total amount)}$$

So 35 ml minus 0.1 ml equals 34.9 ml. This (34.9 ml) is the amount of NRS 1:400 needed for each tube of 1st AB 1:400 dilution to give 1:140,000 dilution.

TABLE A-20. TITER CHECK

*** 1% EGG ALBUMIN was used.

1st AB - LH-B-225 (NISWENDER)
2nd AB - SARGPGG #96
4/2/81
N. Carolina

Total counts - 52,357

First Antibody Dilutions	2nd Antibody Dilutions					
	1:2	1:4	1:8	1:10	1:15	1:20
NSB[A] (No first antibody)	386	425	382	390	386	437
1:160,000	5,748	5,099	6,085	5,622	6,340	-
% Bd	10.9	9.7	11.6	10.7	12.1	-
1:140,000	6,904	5,933	7,082	6,360	7,635	8,301
% Bd	13.2	11.3	13.5	12.1	14.6	15.8
1:120,000	8,030	6,919	7,827	7,062	8,587	9,263
% Bd	15.3	13.2	14.9	13.5	16.4	17.7
1:100,000	9,448	7,883	9,031	8,214	9,957	10,611
% Bd	18.0	15.0	17.2	15.7	19.0	20.2
1:80,000	11,981	9,549	10,882	10,009	11,841	12,804
% Bd	22.8	18.0	20.7	19.1	22.6	24.4
1:60,000	15,321	12,165	13,718	12,822	14,781	15,843
% Bd	29.2	23.2	26.0	24.5	28.2	30.2
NSB[B]	371	262	253	206	217	315

A - flint tubes were used
B - borosilicate tubes
Bd - binding

There is no difference in using flint or borosilicate tubes if egg albumin is used.

TABLE A-21. TITER CHECK

** 1% Bovine Serum Albumin (1% BSA) was used.

1st AB - LH-B-225 - (anti-bovine) Niswender (1982)
2nd AB - SARGPGG #96 - 4/2/81 N. Carolina
+ polyethylene glycol (PEG) 6%

Total Counts = 54,935

First Antibody Dilution	2nd antibody dilution			
	1:20	1:25	1:30	1:35
NSB[A] (no first antibody)	828	767	803	770
1:140,000	11,758	11,998	12,564	12,498
% Bd	21.0	21.8	22.8	22.7
1:120,000	13,479	13,655	14,121	14,308
% Bd	24.0	24.8	25.7	26.0
NSB[B]	3,649	3,000	3,647	3,288

A - borosilicate tubes were used
B - flint tubes were used
Bd - binding

There is difference in NSB due to type of tube used, when 1% BSA is used.

If 1% EA is used, either type of tube can be used.

If 1% BSA is used, only borosilicate tube can be used.

8. Preparation of standard curve in triplicate.
    a. Total counts (TC)

       Add 100 ul of LH^{125}, 50,000 CPM (only LH^{125})
    b. Nonspecific binding (NSB).

       Add 200 ul of NRS 1:400 plus 500 ul of 1% BSA plus 100 ul of LH^{125} plus 700 ul of 2nd AB-PEG 6% (no 1st AB)
    c. Tubes zero (% binding).

       Add 500 ul of 1% BSA plus 200 ul of 1st AB plus 100 ul of LH^{125} plus 700 ul of 2nd AB-PEG 6%
    d. Standard tubes.

       The concentrations are:

       .06, .12, .25, .5, 1, 2.5, 5, 10 ng of LH/ml (see table A-22)
9. Add 200 ul of 1st AB to all tubes except total counts. Shake the tubes.
10. Incubate at 4° C for 24 h.
11. Add 100 ul of LH^{125}. Shake the tubes.
12. Incubate at 4° C for 24 h.
13. Add 700 ul of 2nd AB-PEG 6% to one rack (80 tubes) at a time and then shake the tubes. After finishing all the racks, wait for 15 min.
14. Centrifuge for 30 min at 4° C at 2,800-3,000 RPM.
15. Decant supernatant - have the tubes inverted about 30 minutes over absorbent paper to dry out.
16. Count tubes for 0.5 min (gamma counter).
17. Save standard curve tubes and recount at the end of assay, if there is a large number of tubes.

TABLE A-22. STANDARD CURVE DILUTION

Tube	no.	Volume of standard (ul)	1% BSA (ul)	1st AB (ul)	LH^{125} (ul)	2nd AB-PEG 6% (ul)
TC	3	--	--	--	100	--
NSB	3	200	500	--	100	700
	(NRS 1:400)					
zero	3	--	500	200	100	700
.06*	3	12.5	487.5	200	100	700
.12*	3	25	475	200	100	700
.25*	3	50	450	200	100	700
.50*	3	100	400	200	100	700
1**	3	20	480	200	100	700
2.5**	3	50	450	200	100	700
5**	3	100	400	200	100	700
10**	3	200	300	200	100	700
OVX	3	100	400	200	100	700
OVX	3	200	300	200	100	700
OVX	3	300	200	200	100	700
OVX+LH	3	100	400	200	100	700
OVX+LH	3	200	300	200	100	700
OVX+LH	3	300	200	200	100	700
Unknown	1	100	400	200	100	700
	1	200	300	200	100	700

* Standard solution used is 0.5 ng/100 ul.
** Standard solution used is 5 ng/100 ul.

Validation of LH Assay

1. Different amounts of LH (USDA-bLH-B-5) at a concentration of 5 ng/100 ul were added to a plasma of a cycling Jersey cow on day 12 of the estrous cycle. The amounts used in the validation were:

   0.0, 0.25, .5, 1, 2, 4, 6, 8, 10 and 12 ng/ml (tables A-23 and A-24).

TABLE A-23. PREPARATION OF DIFFERENT CONCENTRATIONS OF STANDARD (USDA-bLH-B-5) IN PLASMA FOR THE VALIDATION OF LH ASSAY

Concentration	Stock	Amount of plasma	Amount of 5 ng/100 ul	Amount and Stock
12 ng/ml	I	1,520 ul +	480 ul	--
10 ng/ml	II	1,600 ul +	400 ul	--
8 ng/ml	III	1,680 ul +	320 ul	--
6 ng/ml	IV	500 ul +	--	500 ul of I
4 ng/ml	V	1,000 ul +	--	1,000 ul of III
2 ng/ml	VI	500 ul +	--	500 ul of V
1 ng/ml	VII	1,800 ul +	--	200 ul of II
.5 ng/ml	VIII	500 ul +	--	500 ul of VII
.25 ng/ml	IX	1,500 ul +	--	500 ul of VII

TABLE A-24. MEASUREMENT OF DIFFERENT AMOUNTS OF LH (USDA-bLH-B-5) ADDED TO A PLASMA

Amount added (ng/ml)	Volume assayed (ul)	Amount measured (ng/ml)	Mean per volume assayed (ng/ml)	Mean per amount added (ng/ml)
0	100	.4		
0	100	.2	.30	
0	100	.3		.33
0	200	.25		
0	200	.45	.36	
0	200	.4		
.25	100	.6		
.25	100	.6	.56	
.25	100	.5		.55
.25	200	.5		
.25	200	.5	.55	
.25	200	.65		
.50	100	.7		
.50	100	.7	.63	
.50	100	.5		.65
.50	200	.85		
.50	200	.75	.68	
.50	200	.45		
1	100	1.1		
1	100	1.3	1.40	
1	100	1.8		1.26
1	200	1.2		
1	200	1	1.11	
1	200	1.15		
2	100	2.3		
2	100	2.3	2.30	
2	100	2.3		2.25
2	200	2.05		
2	200	2.2	2.20	
2	200	2.35		
4	100	4.5		
4	100	4.5	4.46	
4	100	4.4		4.28
4	200	4.15		
4	200	4.05	4.10	
4	200	4.15		
6	100	6.3		
6	100	6.2	6.30	
6	100	6.4		6.05
6	200	5.55		
6	200	6.1	5.81	
6	200	5.8		
8	100	8.6		
8	100	8.6	8.56	
8	100	8.5		8.08
8	200	7.75		

Table A-24-continued.

8	200	7.55	7.61	
8	200	7.55		
10	100	10.9		
10	100	10.5	10.53	
10	100	10.2		9.73
10	200	9		
10	200	10.55	8.93	
10	200	7.25		
12	100	12.4		
12	100	13.2	12.66	
12	100	12.4		11.77
12	200	10.6		
12	200	10.97	10.88	
12	200	11.1		

2. Assaying different volumes of a plasma with high LH content.

A plasma known to have a high concentration of LH was assayed at different volumes. The volumes from 25 ul to 150 ul were completed to 200 ul with a plasma of low LH content (Table A-25).

TABLE A-25. SERIAL DILUTION OF PLASMA FOR MEASUREMENT OF LH

Volume (ul) of plasma of high concentration of LH	Volume (ul) of plasma of low concentration of LH	Amount of LH measured (ng/ml)	Mean per volume assayed	Overall mean
300	0	25.57	22.49	21.95
300	0	20.75		
300	0	21.17		
200	0	22.55	22.97	
200	0	23.10		
200	0	23.25		
150	50	17.70	20.36	
150	50	21.67		
150	50	21.67		
100	100	22.70	22.73	
100	100	23.50		
100	100	22.00		
50	150	21.60	21.67	
50	150	23.00		
50	150	20.40		
25	175	22.00	21.47	
25	175	20.40		
25	175	22.00		
0	50	0.40	0.20	0.253
0	50	0.20		
0	50	0.00		
0	100	0.30	0.30	
0	100	0.30		
0	100	0.30		
0	200	0.30	0.25	
0	200	0.20		
0	200	0.25		
0	300	0.27	0.26	
0	300	0.23		
0	300	0.27		

TABLE A-26. NUTRIENT REQUIREMENT (kg) CALCULATIONS

	DM	CP	TDN
Cow one[a]	9.3	.86	5.0
Cow two[b]	8.5	.78	4.5

[a] live weight 450 kg or 97.7 $kg^{.75}$ (NRC, 1976).
[b] live weight 397 kg or 88.7 $kg^{.75}$ (requirements calculated based on one cow)

For calculations, it was assumed that cows consumed 6 kg of DM of hay per day. The DM of hay and concentrate were 90 and 87%. The amount of concentrate was calculated to supply the difference between the CP required minus CP supplied by 6 kg of hay (DM). The content of TDN supplemented was higher than required.

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I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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